

FORM 100-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

07039-278001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

09/980526

INTERNATIONAL APPLICATION NO.
PCT/US00/14296INTERNATIONAL FILING DATE
24 May 2000PRIORITY DATE CLAIMED
24 May 1999

TITLE OF INVENTION

METHODS TO INHIBIT INFECTIOUS AGENT TRANSMISSION

APPLICANT(S) FOR DO/EO/US

Mark J. Federspiel

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
- ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
- ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ has been communicated by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
- ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
- ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☒ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/ISA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Copy of Request with one substitute pg. (4 pgs.);
Preliminary Amendment (6 pgs.); check and postcard

Date of Deposit 15 November 2001

I hereby certify under 37 CFR 1.10 that this correspondence is being deposited with the United States Postal Service as "Express Mail Post Office To Addressee" with sufficient postage on the envelope indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20514.

U.S. APPLICATION NO. (UNKNOWN) SEE 37 CFR

INTERNATIONAL APPLICATION NO.

ATTORNEY'S DOCKET NUMBER

097980326

PCT/US00/14296

07039-278001

24. The following fees are submitted.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

- ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00
- ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00
- ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00
- ☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

CALCULATIONS PTO USE ONLY

\$860.00

Surcharge of \$130.00 for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)). ☐ 20 ☒ 30

\$130.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	18 - 20 =	0	x \$18.00
Independent claims	6 - 3 =	3	x \$80.00

\$0.00

\$240.00

Multiple Dependent Claims (check if applicable). ☐

\$0.00

TOTAL OF ABOVE CALCULATIONS =

\$1,230.00

☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.

\$615.00

SUBTOTAL =

\$615.00

Processing fee of \$130.00 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)). ☐ 20 ☐ 30 +

\$0.00

TOTAL NATIONAL FEE =

\$615.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable). ☐

\$0.00

TOTAL FEES ENCLOSED =

\$615.00

Amount to be:

refunded \$

charged \$

- a. ☒ A check in the amount of \$615.00 to cover the above fees is enclosed.
- b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.
- c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.
- d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Mark S. Ellinger
Fish & Richardson P.C., P.A.
60 South Sixth Street, Suite 3300
Minneapolis, Minnesota 55402

SIGNATURE

J. Patrick Finn, III

NAME

44,109

REGISTRATION NUMBER

15 November 2001

DATE

#4/B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mark J. Federspiel
Serial No. : 09/980,526
Filed : November 15, 2001
Title : METHODS TO INHIBIT INFECTIOUS AGENT TRANSMISSION

Art Unit : Unknown
Examiner : Unknown

BOX PCT

Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

In response to the communication dated February 1, 2002 (copy enclosed), applicant submits herewith a Sequence Listing in computer readable form as required by 37 CFR §1.824. In addition, applicant submits a substitute Sequence Listing as required under 37 CFR §1.823(a) and a statement under 37 CFR §1.821(f).

Applicant respectfully requests entry of the paper copy and computer readable copy of the Sequence Listing filed herewith for the instant application. Furthermore, applicant requests entry of the following amendments.

In the specification:

Replace the original Sequence Listing with the substitute Sequence Listing filed herewith.


Replace the paragraph beginning at page 38, line 22, with the following rewritten paragraph:

--RT-PCR. RNA is isolated from virions with RNA STAT 50-LS or STAT-60 (Tel-Test, Inc., Friendswood, TX), and converted to cDNA with 2.5 μ M random hexanucleotide primers

CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EL734686785US

I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

April 1, 2002
Date of Deposit
Signature 

Vince Defante
Typed or Printed Name of Person Signing Certificate

and Superscript II (Life Technologies, Gaithersburg, MD) (Wilson et al., 1998). cDNA templates are amplified with primers corresponding to the PERV *pol/env* gene boundary (ACCTCGAGACTCGGTGGAAGGG; SEQ ID NO:14) and the untranslated region 3' of the PERV *env* gene (CTGGGTTCTGGGAGGGTTAGGTTG; SEQ ID NO:15), or amplified with PB906 (5' ACGTACTGGAGGAGGGTCACCTGA 3'; SEQ ID NO:16) and PB908 (5' GTCCCGAACCCCTTATAACCTCTTG 3'; SEQ ID NO:17) or PERVenv1 (sense) (5' ACCTCGAGACTCGGTGGAG; SEQ ID NO:33) and PERVenv2 (anti-sense) (5' CTGGGTTCTGGGAGGGTTAGGTTG; SEQ ID NO:34) for 30 cycles at 94°C for 30 seconds, at 60°C for 30 seconds, and at 72°C for 1 minute. The amplified products are cloned into the PCR II T-A vector (Invitrogen Corp., San Diego, CA).--

REMARKS

Applicant hereby submits that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

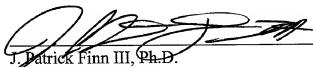
Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date:

April 1, 2002


J. Patrick Finn III, Ph.D.
Reg. No. 44,109

Fish & Richardson P.C., P.A.
60 South Sixth Street
Suite 3300
Minneapolis, MN 55402
Telephone: (612) 335-5070
Facsimile: (612) 288-9696

"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 38, line 22, has been amended as follows:

RT-PCR. RNA is isolated from virions with RNA STAT 50-LS or STAT-60 (Tel-Test, Inc., Friendswood, TX), and converted to cDNA with 2.5 μ M random hexanucleotide primers and Superscript II (Life Technologies, Gaithersburg, MD) (Wilson et al., 1998). cDNA templates are amplified with primers corresponding to the PERV *pol/env* gene boundary (ACCTCGAGACTCGGTGGAAGGG; SEQ ID NO:14) and the untranslated region 3' of the PERV *env* gene (CTGGGTTCTGGGAGGGTTAGGTTG; SEQ ID NO:15), or amplified with PB906 (5' ACGTACTGGAGGAGGGTCACCTGA 3'; SEQ ID NO:16) and PB908 ([15] 5' GTCCCGAACCCCTTATAACCTCTTG 3'; SEQ ID NO:17) or PERVenv1 (sense) (5' ACCTCGAGACTCGGTGGAG; SEQ ID NO:[11] 33) and PERVenv2 (anti-sense) (5' CTGGGTTCTGGGAGGGTTAGGTTG; SEQ ID NO:[12] 34) for 30 cycles at 94°C for 30 seconds, at 60°C for 30 seconds, and at 72°C for 1 minute. The amplified products are cloned into the PCRII T-A vector (Invitrogen Corp., San Diego, CA).

09/980526

Art Unit's Docket No. 07039-278001

NOT RECORDED 15 NOV 2001

#2/a

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mark J. Federspiel

Art Unit : Unknown

Serial No. :

Examiner : Unknown

Filed : Herewith

Title : METHODS TO INHIBIT INFECTIOUS AGENT TRANSMISSION

BOX PCT

Commissioner for Patents

Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, please amend the application as follows:

In the claims:

Please cancel claims 1-45 without prejudice.

Please add claims 46-63 as follows:

--46. A method for reducing transmission of an infectious agent from a mammalian transplant recipient to a donor cell, said method comprising introducing a nucleic acid molecule into a cell such that, when said cell is transplanted into said mammalian transplant recipient thereby being said donor cell, transmission of said infectious agent from said mammalian transplant recipient to said donor cell is reduced.

47. The method of claim 46, wherein said nucleic acid molecule comprises a promoter operably linked to a nucleic acid segment encoding at least a portion of a receptor for said infectious agent.

CERTIFICATE OF MAILING BY EXPRESS MAIL

Express Mail Label No. EL589641942US

I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

Date of Deposit 15 November 2001Signature 20

Signature

Typed or Printed Name of Person Signing Certificate Vince Defante

48. The method of claim 46, wherein said nucleic acid molecule comprises a promoter operably linked to a nucleic acid segment encoding at least a portion of a polypeptide of said infectious agent.

49. The method of claim 48, wherein said infectious agent is a virus.

50. The method of claim 49, wherein said polypeptide is a surface glycoprotein.

51. The method of claim 46, wherein said nucleic acid molecule comprises a promoter operably linked to a nucleic acid segment encoding a fusion protein, wherein said fusion protein comprises:

- a) a first polypeptide segment comprising at least a portion of a polypeptide of said infectious agent, and
- b) a second polypeptide segment comprising degradative enzymatic activity.

52. The method of claim 51, wherein said degradative enzyme activity is a nuclease, protease, or lipase activity.

53. A method for reducing transmission of an infectious agent from a mammalian transplant recipient to a donor cell, said method comprising:

- a) introducing a nucleic acid molecule into a cell such that, when said cell is transplanted into said mammalian transplant recipient thereby being said donor cell, transmission of said infectious agent from said mammalian transplant recipient to said donor cell is reduced, and
- b) transplanting said cell into said mammalian transplant recipient such that said cell is said donor cell.

54. A method for reducing transmission of an infectious agent from a mammalian transplant recipient to a donor cell, said method comprising:

- a) providing a cell,

b) introducing a nucleic acid molecule into said cell such that, when said cell is transplanted into said mammalian transplant recipient thereby being said donor cell, transmission of said infectious agent from said mammalian transplant recipient to said donor cell is reduced, and

c) transplanting said cell into said mammalian transplant recipient such that said cell is said donor cell, and transmission of said infectious agent from said mammalian transplant recipient to said donor cell is reduced.

55. A method for reducing transmission of an infectious agent from a donor cell to a mammalian transplant recipient, said method comprising introducing a nucleic acid molecule into a cell such that, when said cell is transplanted into said mammalian transplant recipient thereby being said donor cell, transmission of said infectious agent from said donor cell to said mammalian transplant recipient is reduced.

56. The method of claim 55, wherein said nucleic acid molecule comprises a promoter operably linked to a nucleic acid segment encoding at least a portion of a receptor for said infectious agent.

57. The method of claim 55, wherein said nucleic acid molecule comprises a promoter operably linked to a nucleic acid segment encoding at least a portion of a polypeptide of said infectious agent.

58. The method of claim 57, wherein said infectious agent is a virus.

59. The method of claim 58, wherein said polypeptide is a surface glycoprotein.

60. The method of claim 55, wherein said nucleic acid molecule comprises a promoter operably linked to a nucleic acid segment encoding a fusion protein, wherein said fusion protein comprises:

a) a first polypeptide segment comprising at least a portion of a polypeptide of said infectious agent, and

b) a second polypeptide segment comprising degradative enzymatic activity.

61. The method of claim 60, wherein said degradative enzyme activity is a nuclease, protease, or lipase activity.

62. A method for reducing transmission of an infectious agent from a donor cell to a mammalian transplant recipient, said method comprising:

a) introducing a nucleic acid molecule into a cell such that, when said cell is transplanted into said mammalian transplant recipient thereby being said donor cell, transmission of said infectious agent from said donor cell to said mammalian transplant recipient is reduced, and

b) transplanting said cell into said mammalian transplant recipient such that said cell is said donor cell, and transmission of said infectious agent from said donor cell to said mammalian transplant recipient is reduced.

63. A method for reducing transmission of an infectious agent from a donor cell to a mammalian transplant recipient, said method comprising:

a) providing a cell,

b) introducing a nucleic acid molecule into said cell such that, when said cell is transplanted into said mammalian transplant recipient thereby being said donor cell, transmission of said infectious agent from said donor cell to said mammalian transplant recipient is reduced, and

c) transplanting said cell into said mammalian transplant recipient such that said cell is said donor cell, and transmission of said infectious agent from said donor cell to said mammalian transplant recipient is reduced.--

Applicant : Mark J. Federspiel
Serial No. :
Filed : Herewith
Page : 5

Attorney's Docket No.: 07039-278001

REMARKS


Claims 1-45 have been canceled without prejudice, and claims 46-63 have been added herein. Thus, claims 46-63 are pending.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be examined. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: November 15, 2001



J. Patrick Finn III, Ph.D.
Reg. No. 44,109

Fish & Richardson P.C., P.A.
60 South Sixth Street
Suite 3300
Minneapolis, MN 55402
Telephone: (612) 335-5070
Facsimile: (612) 288-9696

Applicant : Mark J. Federspiel
Serial No. :
Filed : Herewith
Page : 6

Attorney's Docket No.: 07039-278001

Version with markings to show changes made

In the claims:

Please cancel claims 1-45 without prejudice.

07039-278001

METHODS TO INHIBIT INFECTIOUS AGENT TRANSMISSION DURING XENOTRANSPLANTATION

5 **Background of the Invention**

- The success of solid organ transplantation protocols continues to improve while the availability of donor organs remains limited, leading to a critical shortage (Policy et al., 1996). In 1995, only one-half of the patients on organ transplant waiting lists received transplants. Approximately nine patients per day died while waiting for a suitable organ to become available.
- 10 Xenotransplantation of animal organs is under consideration as a supplemental approach to alleviate this shortage (Policy et al., 1996). The pig is currently the donor of choice for cells, tissues and vascularized organs used in permanent and transient xenotransplantation treatments (Fishman, 1994). Swine offer a number
- 15 of advantages as a source for xenotransplantations: economic and animal husbandry issues, the ability to produce transgenic animals, experience as a transplantation model, and the development of specific pathogen-free herds to eliminate known pathogens.
- Pig to human organ transplants are discordant. However, several groups
- 20 have engineered transgenic pig lines in efforts to control hyperacute rejection (HAR) (Lin et al., 1996). Two immunologic factors initiate HAR of xenografts: the interaction of natural xenoreactive antibodies to the Gal α 1-3Gal carbohydrate (α -Gal) of discordant cell proteins; and the inability of the xenograft complement regulatory proteins to control the recipient's complement
- 25 system. Several approaches to control HAR are being studied including the ablation of Gal α 1-3Gal expression (Sandrin et al., 1995), and the design of transgenic pigs which express complement regulatory proteins (e.g., DAF, CD59) which have been shown to be protective against HAR (Diamond et al., 1996; Foder et al., 1994; McCurry et al., 1995; Oldham et al., 1996; Ryan,
- 30 1995).

Several promising xenotransplantation approaches use pig cells and tissues in protocols where xenotic cells and tissues are not rejected as readily as whole organs: fetal pig neuronal cells producing dopamine have been tested in patients to replace cells destroyed by Parkinson's disease (Deacon et al., 1997);

and fetal pig pancreatic islet cell clusters have been tested in protocols to treat diabetes (Groth et al., 1994). Another xenotransplantation approach has used pig liver and kidney xenografts as temporary support treatments for patients with fulminant organ failure (Breimer et al., 1996; Groth et al., 1994; Makowka et al., 1995). These studies have sparked research in the use of bioartificial organs and whole organ perfusion using xenogeneic tissue for bridging patients prior to transplantation. The early successes of these innovative clinical approaches indicate that xenotransplantation protocols using pig cells, tissues and organs will continue regardless of the results of solid organ xenotransplantation.

Besides the expected immunological hurdles that must be overcome, xenotransplantation produces a risk of transmitting infectious disease (xenoses) from the source animal tissue to the recipient, and possibly to the recipient's contacts. Xenotransplantation increases the risk of transferring infectious agents from pigs to humans due to the breach of the normal physical barriers against infectious agents upon introduction of pig cells, tissues or organs; the therapeutic immunosuppression of the recipient to prevent graft rejection which interferes with the mechanisms which defend human cells from infection by zoonotic viruses; and the genetic modifications of pigs, e.g., those being tested to control HAR or modify complement activation, may allow zoonotic viruses to evade immune surveillance or prevent the inactivation of invading enveloped viruses (Bergelson et al., 1995; Dorig et al., 1993; Fishman, 1994; Ward et al., 1994; Weiss, 1998).

Since most vertebrates carry endogenous retroviruses in their germline DNA (Boeke et al., 1997), endogenous retroviruses in xenografts may be an infectious risk to xenotransplant patients. The risks of transmission to humans with endogenous viruses of non-humans are unknown and difficult to assess. Endogenous retroviruses from a variety of animals, including baboons, cats, and mice, can infect human cells (Boeke et al., 1997). The ability of endogenous murine leukemia viruses (MLVs) to be activated and infect human tumor xenografts in immunodeficient mice is well documented (Achong et al., 1976; Beattie et al., 1982; Crawford et al., 1979; Gautsch et al., 1980; Suzuki et al., 1977; Tralka et al., 1983; Wunderi et al., 1979). The observation that the gibbon ape leukemia virus SEATO, a virus most likely derived from an endogenous

retrovirus of Asian wild mice, can cause chronic myelogenous leukemia in normal juvenile gibbon apes highlights the potential risks of exposure to xenotic endogenous retroviruses (Kawakami et al., 1980; Lieber et al., 1975).

There have been several reports of MLV retroviral vector stocks that contain the result from recombination between the vector and endogenous retroviruses in the genome of the helper cell line (Chong et al., 1998; Donahue et al., 1992; Patience et al., 1996; Purcell et al., 1996; Vanin et al., 1994). In one study, 3 of 10 rhesus monkeys developed T-cell lymphomas after autologous transplantation of enriched bone marrow stem cells transduced with a MLV retroviral vector stock containing an array of MLV-related recombinant viruses (Vanin et al., 1994). Finally, the activation of endogenous retrovirus by human tumor xenografts as well as studies of graft versus host reaction *in vivo* and mixed lymphocyte reaction *in vitro* (Hirsch et al., 1970; Hirsch et al., 1972; Levy et al., 1977; Sherr et al., 1974), demonstrate that normally inactive endogenous retroviruses can be induced by xenotransplantation conditions.

Pig endogenous retroviruses (PERVs) released by established pig cell lines were observed in the 1970s, but the host range of these viruses was thought to be restricted to pig cells (Armstrong et al., 1971; Lieber et al., 1975; Moennig et al., 1974; Strandstrom et al., 1974; Todaro et al., 1974). Recently, replication-competent pig endogenous retroviruses (PERV) were identified, and shown to be capable of productively infecting human cells *in vitro* (Wilson et al., 1998; Martin et al., 1998; Patience et al., 1997; Takeuchi et al., 1997; Weiss, 1997). PERVs have been associated with pig lymphomas (Bostock et al., 1973; Frazier, 1985; Moennig et al., 1974; Strandstrom et al., 1974; Suzuka et al., 1985). Although Heneine et al. (1998) and Patience et al. (1998) screened blood lymphocytes and sera from 10 diabetic patients that received porcine fetal islet cells, and 2 renal dialysis patients whose circulation had been linked with pig kidneys extracorporeally, respectively, neither group detected PERVs. However, the risk of viral infection is increased in transplantation by the presence of factors commonly associated with viral activation, e.g., immune suppression, graft-versus-host disease, graft rejection, viral coinfection and cytotoxic therapies. Thus, in immunosuppressed xenotransplant patients, there may be a greater risk that infection would lead to disease.

Therefore, there is a need for a method to inhibit or prevent transmission of infectious agents, from donor to recipient and/or recipient to donor, after cell, tissue or organ transplant.

Summary of the Invention

5 The invention provides isolated and purified nucleic acid molecules and methods useful to inhibit or prevent the transmission of an infectious agent during xenogeneic or allogeneic cell, tissue or organ transplant. Infectious agents include, but are not limited to, viruses, bacteria and parasites. The invention includes prophylactic and therapeutic uses of the nucleic acid molecule
10 of the invention which encodes at least a portion of a polypeptide of the infectious agent or which encodes at least a portion of the receptor employed by the infectious agent to enter the cells of the transplant recipient. It is preferred that the expression of the nucleic acid molecule in a donor cell, tissue or organ inhibits or prevents the transmission of the infectious agent from the donor cells,
15 tissue or organ to a transplant recipient, or, alternatively, the transmission of the infectious agent from the recipient to the donor cell, tissue or organ. Preferred transplant recipients are mammals such as primates, e.g., humans, apes and monkeys, as well as canines, felines, bovines, ovines, swine, and equines.

 Preferably, the transmission of the following infectious agents is
20 inhibited or prevented: retroviruses, lentiviruses, herpesvirus, e.g., cytomegalovirus (CMV) and Epstein Barr virus (EBV), and hepatitis viruses, e.g., hepatitis A, B or C. Thus, transplanted organs, e.g., bioengineered organs, tissues, or cells, e.g., stem cells, including allografts, e.g., human-to-human transplants, or xenografts, are genetically modified to inhibit the infection of
25 several major infectious pathogens that limit the success of transplantation (e.g., CMV, EBV, HIV and hepatitis viruses) or the infection by potential pathogens, e.g., endogenous viruses such as PERVs. Preferred nucleic acid molecules useful in the practice of the invention include a nucleic acid molecule which encodes gag, pol, env, protease, and accessory proteins (e.g., Vpr, Vif, Nef, Tat,
30 or Rev) of retroviruses, lentiviruses and spumaviruses; a nucleic acid molecule which comprises a capsid or envelope gene of a herpesviruses, e.g., HSV 1 and 2, EBV and CMV; a nucleic acid molecule which comprises the C gene (nucleocapsid), ORFS/pre-S gene (viral surface glycoproteins), ORFX gene

(regulatory protein), or ORFP gene (viral polymerase) of Hepadnaviruses, e.g., hepatitis B virus; a nucleic acid molecule which comprises the C gene (viral capsid) or the E1 and/or E2 gene of Flaviviruses, e.g., hepatitis C virus; a nucleic acid molecule which comprises ORF1, e.g., which encodes Pro (protease), the X gene, the H gene (helicase), the Pol gene (polymerase), or the ORF2 gene, which codes for capsid, of hepatitis E virus; and a nucleic acid molecule which encodes the P1 gene, the 1A gene, the 1B gene, the 1C gene, or the 1D gene (capsid and glycoproteins) of hepatitis A virus. The alteration of human cells and tissue is preferred for modifying pluripotent cells and in *ex vivo* gene therapy protocols.

Preferably, viral entry and/or assembly is inhibited. To inhibit viral entry, receptor interference may be employed. Receptor interference refers to the expression of a receptor protein for the infectious agent or the ligand thereof, e.g., a viral ligand such as a viral glycoprotein. Preferably, the genome of the donor cell, tissue or organ is augmented with DNA encoding the viral ligand thereby preventing infection. Alternatively, or in addition, capsid-targeted viral inactivation may be employed to inhibit or prevent viral replication. Capsid-targeted viral inactivation (CTVI), reduces or eliminates the production of infectious virus by incorporating a degradative enzyme into newly synthesized viral particles, e.g., by expressing a fusion polypeptide comprising a viral polypeptide and a degradative enzyme. Degradative enzymes such as nucleases, e.g., RNase H, staphylococcal nuclease or ribozymes (see Marshall et al., 1994, and U.S. Patent No. 5,811,275), lipases or proteases, may be employed in the practice of the invention. Preferred fusion polypeptides include fusions of viral capsid, envelope or accessory proteins such as Vif, Vpx, Vpr and Nef, with degradative enzymes.

A preferred xenograft for use in the methods of the invention is swine neuronal cells, pancreatic islet cells, hepatocytes, heart, liver or kidney. Therefore, the invention provides a method in which the genomic DNA of swine cells, tissue or organ to be transplanted is augmented with a recombinant DNA molecule encoding a polypeptide of an infectious agent which binds to a cell surface receptor of a transplant recipient, e.g., a viral glycoprotein, or a fusion polypeptide comprising at least a portion of an infectious agent polypeptide and

a degradative enzyme. Infectious agents in this embodiment of the invention include, but are not limited to, those described in Table 1 of Fishman (1994), which is specifically incorporated by reference herein. In particular, PERVs are useful in CTVI methods to inhibit or prevent human-tropic PERV infection in xenotransplant patients.

As described hereinbelow, human tropic PERVs are produced by activated peripheral blood mononuclear cells. The relative PERV proviral copy number doubled upon passaging of the 1°-293 PERV-infected cells to fresh 293 cells, indicating that passaging the virus may amplify the replication-competent PERV. Only PERV-A *env* sequences were detected in DNA isolated from all three 293/PERV infected cultures by PCR analysis with PERV-A, PERV-B and PERV-C specific *env* primers. The 1°-293/PERV DNA contained a low level of PERV-C *env*. PERV-B *env* was not detected in any culture. Characterization of these PERVs indicated that human-tropic PERVs may be more diverse than previously thought. For example, significant sequence differences were found in the cloned LTR and *env* sequences compared to known PERV sequences. The 293/PERV LTRs contained 71 additional nucleotides compared to the PERV-MSL LTR, and the envelope amino acid sequence contains a PERV-A surface region (SU) sequence but a PERV-C transmembrane (TM) region. These PERVs are useful to determine the tissue tropism of various isolates and to determine the prevalence of anti-PERV antibodies in humans exposed to sources of PERV, e.g., pig slaughterhouse workers and xenotransplant patients such as those having a bioartificial liver with pig hepatocytes, as well as in the methods of the invention.

Thus, a vector comprising a nucleic acid molecule of the invention may also be employed to deliver soluble *env* genes to mammalian donor cells, tissues or organs prior to cell, tissue or organ transplantation so that the transplanted cells, tissues or organs are less susceptible to infection by an infectious agent, the genome of which comprises the *env* gene. For example, soluble PERV *env* genes are introduced to donor swine cells, tissues or organs to inhibit infection of the cells, tissues or organs by PERV, e.g., to inhibit infection by PERVs after the virus is reactivated following transplantation.

As also described hereinbelow, two genes encoding an avian leukosis virus (ALV) receptor protein, sTva proteins, *stva* and *stva-mIgG*, were efficiently delivered and broadly expressed by ALV-based retroviral vectors both in cultured cells and in chickens. Both the sTva and sTva-mIgG proteins significantly inhibited ALV(A) infection *in vitro* and *in vivo*. The antiviral effect was specific for ALV(A), consistent with a receptor interference mechanism.

Brief Description of the Figures

Figure 1. Stimulated pig peripheral blood mononuclear cells (PBMCs) produce replication-competent human-tropic PERV. (A) Porcine PBMC were cultured with either phytohemagglutinin (PHA) + phorbol myristate acetate (PMA) (black bars) or PMA + calcium ionophore (grey bars) and the supernatants assayed for RT activity. (B) NIH and Yucatan minipig PBMC stimulated by PHA + PMA were cocultured with ST-IOWA cells. Cell culture supernatants were assayed for RT activity. Background RT values for uninfected ST-IOWA cells have been subtracted from the values shown. NIH (black squares); Yucatan (open circles). (C) NIH minipig PBMC stimulated with PHA + PMA were cocultured with human 293 cells: 293 cells cocultured with live PBMC (closed squares); with lethally irradiated PBMC (open squares). The cell culture supernatants were assayed for RT activity.

Figure 2. Schematic overview of the generation of the human 293 cell cultures infected with PERV produced by stimulated NIH minipig primary PBMC.

Figure 3. Relative titers of PERV produced from the infected 293 cell cultures. The titer of MLV- β gal/PERV pseudotyped virus stocks produced from the 1^o, 2^o and 3^o-293/PERV cultures were quantitated on human 293 cells (open bars) and pig ST-IOWA cells (black bars). The assays scored β -galactosidase producing cells (BFU) and were done in triplicate.

Figure 4. Analysis of the relative number of PERV proviruses per cell in the 293/PERV infected cultures. Genomic DNA isolated from human 293 cells and the 293/PERV infected cultures was digested with EcoRI, and the fragments separated by agarose gel electrophoresis, transferred to nitrocellulose, and probed with ³²P-labeled PERV *pol* sequences. Two internal proviral DNA fragments hybridized to the PERV *pol* probe as expected. The film was scanned

and the bands quantitated by Image Quant (Molecular Dynamics, Sunnyvale, CA). The blot was stripped and reprobed with ^{32}P -labeled human GAPDH sequences to normalize for the amount of DNA loaded per lane. After the band densities were normalized, the PERV *pol* level of the 1°-293/PERV DNA was set as 1 copy.

Figure 5. RT activity observed in cocultures of 293/2° virus producer cells and human hematopoietic cell lines. The values shown are of cpm of ^3H -TTP incorporated in a reverse transcriptase assay measured in cell supernatants sampled at the times indicated post-coculture of 293/2° virus producer cells with each of the cell lines.

Figure 6. A comparison of the deduced amino acid sequences of the *env* genes of PERV-1.15 (SEQ ID NO:3 encoded by SEQ ID NO:18), PERV-A (Letissier et al., 1997; SEQ ID NO:4) and PERV-C (Akiyoshi et al., 1998; SEQ ID NO:5). A schematic representation of the regions of the PERV-1.15 gene homologous to PERV-A (hatched) and PERV-C (open) genes (top figure). The envelope surface (SU) and transmembrane (TM) glycoprotein regions are also shown. Identical amino acids are denoted by a (·); gaps are denoted (_).

Figure 7. Schematic representation of the cloned PERV sequences. A proposed model for a PERV provirus with estimated locations of the viral domains is shown at the top. The PERV sequences contained in the six unique lambda clones are shown compared to the model. The lamA8 *pol* gene contains a 86 nucleotide deletion (del), and the *env* gene contains a 101 nucleotide insertion (ins) compared to all of the comparable sequences.

Figure 8. A comparison of the nucleotide sequences of two PERV LTRs. The LTR sequence of the published cDNA clone PERV-MSL (SEQ ID NO:7) is compared to the lamA1 LTR sequence (SEQ ID NO:6). Identical bases are denoted by (·); gaps are denoted (_). The estimated location of the major regions of the LTR, U3, R, and U5 are indicated. The sequence of the putative tRNA binding site is also indicated (18 of 18 nucleotides identical to the tRNA^{Pro} binding site).

Figure 9. Schematic representation of the construction of recombinant PERV molecular clones.

Figure 10. Analysis of PERV proteins by SDS-PAGE and Western blot.

(A) Virus was pelleted by ultracentrifugation through a 20% sucrose pad, separated by 12% SDS-PAGE, and visualized by staining with Coomassie brilliant blue. Lane 1, 5 ml 293 supernatant; lane 2, 5 ml 2°-293/PERV supernatant; lane 3, 15 ml 2°-293/PERV supernatant; lane 4, 5 ml DF-1 supernatant; and lane 5, 5 ml avian leukosis virus infected DF-1 supernatant. (B) Viral proteins were prepared and separated by SDS-PAGE as described above, and transferred to nitrocellulose. Lanes 1, 3, 5 and 7, 5 ml 293 supernatant; and lanes 2, 4, 6 and 8, 5 ml 2°-293/PERV supernatant. Lanes 1 and 2 were exposed to 1:1000 dilution of goat anti-GALV capsid antisera; lanes 3 and 4 to 1:1000 goat preimmune sera; lanes 5 and 6 to 1:1000 goat anti-SSAV capsid antisera; and lanes 7 and 8 to 1:1000 goat preimmune sera. The filters were washed and exposed to peroxidase-labeled rabbit anti-goat antibody. The antibody complexes were detected by chemiluminescence, and exposed to film for 30 seconds. The PERV CA protein migrates at about 25 kDa.

Figure 11. Schematic of sTva antiviral gene constructs and the ALV-based retroviral vectors.

Figure 12. General procedure for using replication-competent ALV-based retroviral vector system *in vitro* and *in vivo*.

Figure 13. sTva-mIgG receptor expression levels in DF-1 cells. The sTva-mIgG protein was immunoprecipitated with goat α -mouse-IgG agarose beads from supernatants (500 μ l) of DF-1 cultures infected with the RCASBP(C), RCAS(C) or RCOSBP(C) vectors alone (V) or containing the *sTva-mIgG* gene (sTva). The immunoprecipitates were denatured, separated by 12% SDS-PAGE, and analyzed by Western transfer. The filter was probed with peroxidase-conjugated goat anti-mouse IgG, and the bound protein-antibody complexes were visualized by chemiluminescence on Kodak X-Omat film. sTva-mIgG protein expressed transiently in human embryonic kidney 293 cells was included as a positive control (+).

Figure 14. sTva-mIgG expression in sera of chickens infected with RCASBP vectors. The sTva-mIgG protein was immunoprecipitated from chicken serum (500 μ l) and analyzed as described (Figure 13 legend). Lane 1, uninfected control; Lane 2, a RCASBP(B) vector alone infected bird; Landes 3-

4, RCASBP(B)stva-mIgG infected birds; Lane 5, a RCASBP(C) vector alone infected bird; Lane 6, a RCASBP(C)stva-mIgG infected bird; Lane 7, RCASBP(C)stva-mIgG infected DF-1 cells as a positive control.

Figure 15. Analysis of viral and soluble receptor RNA levels in tissues of chickens infected with RCASBP(B). The figure shows autoradiograms of 6% polyacrylamide/7.6 M urea gels used to separate the protected RNA probe fragments produced in an RNase protection assay with RNA from a bird infected with RCASBP(B)stva or RCASBP(B)stva-mIgG. RNA was prepared from liver (L), heart (H), spleen (S), bursa (B), thymus (T), kidney (K), and muscle (M) tissues of each bird. RNA from DF-1 cells infected with the appropriate virus was included as a positive control (+). RNA from bursa of an uninfected bird was the negative control (-). ALV(B) *env* RNA protects a 467-nt fragment from the 522-nt ³²P-labeled full-length probe [*env*(B)]; *stva* RNA protects a 388-nt fragment from the 498-nt probe (*stva*); and *stva-mIgG* RNA protects a 363-nt fragment from the 423-nt probe (*stva-mIgG*). Each assay contained a chicken GAPDH probe as a control for RNA quality and quantity. GAPDH RNA protects a 200-nt fragment from the 279-nt GAPDH probe.

Figure 16. Analysis of viral and soluble receptor RNA levels in the bursa of birds infected with RCASBP(B) vectors. The figure shows an autoradiogram of a 6% polyacrylamide/7.6 M urea gel used to separate the protected RNA probe fragments produced in an RNase protection assay with RNA from the bursa of birds infected with RCASBP(B) alone (V), RCASBP(B)stva (S), RCASBP(B)stva-mIgG (I), or uninfected (U). RNA from DF-1 cells infected with the appropriate virus was included as a positive control (+). ALV(A) *env* RNA protects a 423-nt fragment from the 483-nt ³²P-labeled full-length probe [*env*(A)]; ALB(B) *env* RNA protects a 467-nt fragment from the 522-nt probe [*env*(B)]; *stva* RNA protects a 388-nt fragment from the 498-nt probe (*stva*); and *stva-mIgG* RNA protects a 363-nt fragment from the 423-nt probe (*stva-mIgG*). Each assay contained a chicken GapDH probe as a control for RNA quality and quantity. GAPDH RNA protects a 200-nt fragment from the 279-nt GAPDH probe. Full-length probes not treated with RNase: lane A, *env*(A); lane B, *env*(B); lane S, *stva*; lane I, *stva-mIgG*; and Lane G, GAPDH.

Figure 17. Analysis of viral and soluble receptor DNA in tissues of RCASBP(B) infected birds and challenged with RAV-1. Genomic DNA was isolated from liver (L), heart (H), spleen (S), bursa (B), thymus (T), kidney (K), and muscle (M) samples of birds infected with RCASBP(B) alone (I),

5 RCASBP(B)stva (II) and RCASBP(B)stva-mIgG (III) and challenged with RAV-1. DNA isolated from DF-1 cells infected with the appropriate virus was used as a positive control (+). DNA isolated from the bursa of an uninfected control bird was used as the negative control (-). DNA sequences were detected by PCR using specific primer pairs: RAV-1 challenge virus DNA [env(A)] was

10 detected using primers specific for ALV(A) *env* yielding a 937 bp fragment; RCASBP(B) vector DNA [env(B)] was detected using primers specific for ALV(B) *env* yielding a 429 bp fragment; *stva* DNA (*stva*) and *stva-mIgG* DNA (*stva-mIgG*) were detected with specific primers yielding fragments of 314 bp and 589 bp respectively. The amplified DNA fragments were separated on 0.8%

15 agarose gels and visualized with ethidium bromide. The molecular weight marker is the 1 Kb plus DNA ladder (Gibco).

Figure 18 A) Nucleotide sequence of *env* gene of PERV 1.15 (SEQ ID NO:18). B) Nucleotide sequence of PERV sequences in lambda A1 clone (SEQ ID NO:19). C) Nucleotide sequence of PERV sequences in lambda A10 clone

20 (SEQ ID NO:20). D) Nucleotide sequence of PERV sequences in lambda A11 clone (SEQ ID NO:32). E) Nucleotide sequence of PERV sequences in lambda A3A clone (SEQ ID NO:21). F) Nucleotide sequence of PERV sequences in lambda A6 clone (SEQ ID NO:22). G) Nucleotide sequence of PERV sequences in lambda clone A8 (SEQ ID NO:23).

25 **Detailed Description of the Invention**

Definitions

As used herein, the terms "isolated and/or purified" refer to *in vitro* preparation, isolation and/or purification of a nucleic acid molecule or polypeptide (e.g., antibody) of the invention, so that it is not associated with *in*

30 *vivo* substances. Thus, with respect to an "isolated nucleic acid molecule", which includes a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, the "isolated nucleic acid molecule" (I) is not associated with all or a portion of a polynucleotide in which the "isolated nucleic acid

molecule" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. An isolated nucleic acid molecule means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA. The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset with 200 bases or fewer in length. Preferably, oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g., for probes; although oligonucleotides may be double stranded, e.g., for use in the construction of a variant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides. The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. An oligonucleotide can include a label for detection, if desired.

The term "isolated polypeptide" means a polypeptide encoded by cDNA or recombinant RNA, or is synthetic origin, or some combination thereof, which isolated polypeptide (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g., free of human proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

The term "sequence homology" means the proportion of base matches between two nucleic acid sequences or the proportion amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length of a sequence that is compared to some other sequence (the reference sequence). Gaps (in either of the two sequences) are permitted to maximize

matching; gap lengths of 15 bases or less are usually used, 6 bases or less are preferred with 2 bases or less more preferred. When using oligonucleotides as probes or treatments, the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches
5 out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and more preferably not less than 19 matches out of 20 possible base pair matches (95%).

The term "selectively hybridize" means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments of the invention
10 selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides,
15 oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest is at least 65%, and more typically with preferably increasing homologies of at least about 70%, about 90%, about 95%, about 98%, and 100%. Preferred PERV nucleic acid sequences for use in the methods of the invention are those which have at least about 80%, more preferably 90%, and
20 even more preferably 95%, contiguous nucleotide sequence homology or identity to SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 or SEQ ID NO:32. Preferably, PERV nucleic acid sequences are those which hybridize under moderate, preferably under stringent, hybridization conditions to SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20,
25 SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 or SEQ ID NO:32.

Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are
30 allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment

score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff, M. O., in *Atlas of Protein Sequence and Structure*, 1972, volume 5, National Biomedical Research Foundation, pp. 101-110, and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program.

The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity.

A "comparison window", as used herein, refers to a conceptual segment of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. (U.S.A.)* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over the window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denote a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window of at least 20-50 nucleotides,

wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

- 5 As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least about 80 percent sequence identity, preferably at least about 90 percent sequence identity, more preferably at least about 95 percent sequence identity, and most preferably at least about 99
10 percent sequence identity.

- Moderate and stringent hybridization conditions are well known to the art, see, for example sections 9.47-9.51 of Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). For example, stringent conditions are those that (1) employ low ionic
15 strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate (SSC); 0.1% sodium lauryl sulfate (SDS) at 50°C, or (2) employ a denaturing agent such as formamide during hybridization, e.g., 50% formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM
20 NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% sodium dodecylsulfate (SDS), and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1%
25 SDS.

CTVI

- The present invention describes a method of interfering with viral replication. For example, the fusion of an enzyme (such as a nuclease, lipase, or protease) to a viral capsid, glycoprotein or accessory polypeptide which is
30 incorporated into virions can inactivate essential viral components, such as nucleic acid, protein or lipid, which are necessary for replication. This technique is referred to as capsid-targeted viral inactivation (CTVI), although it includes the targeting of non-capsid fusions, e.g., env fusions, to the virion as well.

- The present invention uses nucleases, proteases, and/or lipases as antiviral therapeutic agents that degrade or modify essential viral components, such as nucleic acids, proteins, or lipids. The method of the present invention can be applied to viruses that have relatively flexible capsid structures such as
- 5 retroviruses, lentiviruses, herpesviruses, poxviruses, togaviruses, hepadnaviruses, caulimoviruses, myxoviruses and paramyxoviruses, that readily allow the encapsidation of foreign proteins in an aqueous internal compartment containing the viral nucleic acid. Preferred viruses to be inhibited by the methods of the invention include porcine endogenous retroviruses (PERVs),
- 10 herpesviruses such as EBV and CMV, and hepatitis viruses, e.g., hepatitis A, B, and C. See Fields, Virology, Lippincourt-Raven, Philadelphia, PA (1996), which is specifically incorporated by reference herein. The enzyme portion of the fusion protein preferably is oriented so that it is internal to the assembled capsid structure. However, externally facing enzymes may be used in the case of
- 15 proteases and lipases. The use of such constructs does not require the virus to have an aqueous internal compartment. In the case of lipases, it is desirable to target the enzyme to the envelope of the virus. The enzyme portion can be either inside or outside the viral envelope. In this way, any enveloped virus can be targeted by CTVI.
- 20 Retroviruses, and the closely related retrotransposons, which are referred to herein generically as retroviruses, are particularly susceptible to CTVI because of their assembly mechanism. It is possible to take advantage of the knowledge of how these capsids assemble to direct a destructive enzyme molecule to the inside surface of the capsid, where it can contract viral nucleic acids or proteins.
- 25 Retroviral and retrotransposon reverse transcriptases are expressed as natural fusion proteins, in which the polymerase protein forms the C-terminal portion of the primary translation product and the gag protein forms the N-terminal portion. The mechanisms by which Gag-Pol fusion proteins are formed in different retroviruses vary, sometimes involving frameshifting during translation,
- 30 suppression of a nonsense codon, or splicing. Regardless of the mechanism of synthesis, the reverse transcriptase protein sequence is found at the C-terminus of the Gag protein sequences. In addition, the reverse transcriptase is found internal to the capsid structure. Substitution of a destructive enzyme coding

region for the reverse transcriptase gene (i.e., inserting an open reading frame for a destructive enzyme into the *pol* gene in frame), leads to the assembly of the enzyme in retroviral particles. Further, the nuclease or protease is oriented relative to the capsid in such a way as to have access to the viral RNA or reverse transcriptase. Gag-degradative enzyme fusions, as well as gag-pol-degradative enzyme fusions and env-degradative enzyme fusions, are envisioned. Further, the amino acid linkage between the viral proteins and the degradative enzyme may include sequences that are susceptible to proteases present in the virion, and so are cleaved to yield a soluble degradative enzyme.

Thus, a dominant negative effect is obtained by fusion of a protein with a destructive or modifying enzymatic activity that can alter or destroy an essential component of the virus (e.g., DNA, RNA or protein). The destructive or modifying activity is targeted to the desired site of action (inside or closely adjacent to the virus particle) because it is covalently linked to a core protein of the viral particle, e.g., in the same manner as reverse transcriptase.

Besides its versatility, one of the main advantages of CTVI is that it is enzymatic. It does not rely on massive over-expression of a mutant protein that in some way interferes with the viral life cycle, as is the case in the strategies referenced above. Rather, a small number of enzyme molecules (e.g., nucleases) are incorporated into a viral particle, and they cleave or modify one or more essential components of the virus.

CTVI also has advantages over traditional chemotherapy. Viruses, especially viruses with error-prone replicative mechanisms such as HIV and other retroviruses, are likely to become resistant to a chemotherapeutic agent.

For example, HIV isolates resistant to AZT, a widely used anti-HIV drug, have been reported. Clearly, resistance to drugs is an important problem with viral infections just as it is for bacterial infections. Gene therapy or intracellular immunization strategies may also be subject to problems of resistance. For example, if a virus mutates such that the capsid protein no longer assembles with the capsid fusion protein of the present invention, the virus will become resistant to the particular CTVI therapy. However, the DNA of the mutant virus can be cloned and the mutant capsid gene can be fused to the gene encoding a nuclease or other enzyme, thereby restoring the efficacy of the therapy. This approach is

not possible with normal drug therapy; once a virus becomes resistant to a given drug, use of that drug has to be abandoned.

5 Fusion proteins according to the present invention comprise a destructive enzyme which is capable of destroying the replication ability of a virus or virus-like particle. As used in the present invention, "replication ability" encompasses infectivity of a virus particle or transposition of a retrotransposon. Typically, the destructive enzyme is a nuclease, lipase or protease. Protein-modifying enzymes, such as kinases, which diminish enzyme activity can also be used, for example, one which inactivates a viral encoded polymerase. Fusion proteins
10 also comprise a capsid protein, such as Gag, which confers upon the fusion proteins the ability to co-assemble into a virus particle or envelope protein. As mentioned above, it may be desirable in the case of lipase fusions that the virus-encoded portion of the fusion protein be an envelope protein, rather than a capsid protein.

15 Nucleases which can be used in the practice of the present invention to make fusions include restriction endonucleases such as EcoRI, and less specific RNases, such as T1 RNase, barnase, RNase H1, RNase III, retroviral RNase H domains from reverse transcriptase, and staphylococcal nuclease (SN). Other destructive enzymes which can be used in the practice of the present invention
20 include proteases, e.g., nonhomologous retroviral proteases, and lipases. Any such enzymes known in the art can be used. These enzymes degrade proteins and lipids which may be essential for the replication (infectivity) of particular viruses. Other enzymes which modify protein structures may be used, if they result in the inactivation of proteins which are necessary for the viral life cycle.
25 such protein modification enzymes include: kinases, glycosylases, phosphatases, methyl transferases, acetylases, acylases, farnesyl transferases, and demyristylases.

High levels of expression of the fusion proteins are not required, as a single active enzyme molecule can be sufficient to inactivate a virus particle.
30 However, it is desirable that each virus particle formed have at least a single molecule of the fusion protein. Thus, statistical considerations would dictate that expression at a rate greater than one molecule of fusion protein per virus particle be obtained.

In some embodiments of the invention, it may be desirable to utilize conditionally active or marginally active enzymes, since the destructive activity of the enzyme may be deleterious to cells used to prepare the constructs of the present invention. Thus, for example, calcium dependent enzymes may be used, which require a threshold level of calcium for activity. Calcium-dependent destructive enzymes which may be used include calpain, phospholipase A2 and staphylococcal nuclease. It may also be desirable to non-conditionally diminish the activity of the destructive enzyme to a level which is not toxic to cells but which is toxic to viruses. Random mutagenesis can be used to accomplish this end.

DNA molecules are also provided according to the invention, which code for fusion proteins as described above. The DNA molecules comprise appropriate transcriptional and translational control signals which allow the DNA molecules to be expressed in the host cells of the viruses from which the capsid or envelope protein is derived. The expression of the fusion proteins may be dependent upon the presence of virus in the cell.

Recombinant virus is also provided by the present invention. These particles comprise a nucleic acid molecule encoding a fusion protein, as described above. Thus, infection of cells with these recombinant viruses will yield fusion proteins. The recombinant virus is desirably not related to the virus from which the capsid or envelope protein of the fusion protein is derived. The recombinant virus is a mere vehicle for introduction of the fusion constructs. When the cells are infected with viruses which are cognate to the fusion protein capsid or envelope portion, the normal infection cycle of the viruses proceeds. However, when the viruses encapsidate viral nucleic acid, wild-type capsid or envelope proteins co-assemble with fusion proteins. Thus, the newly assembled viruses are hybrids comprising wild-type and fusion capsid or envelope proteins. The newly assembled viruses are not infective because the enzyme portion of the fusion protein inactivates either the viral nucleic acid or the essential viral-packaged proteins or lipid comprising the viral envelope.

Exemplary nucleic acid molecules are those comprising PERV nucleic acid sequences. Thus, for fusion proteins of the invention, it is preferred that nucleic acid molecules encoding PERV capsid or env are employed. For

example, recombinant virus may be obtained from cells, e.g., helper cells, transfected or infected with a recombinant DNA molecule encoding a fusion protein comprising PERV capsid linked to a degradative enzyme. Preferably, once the fusion protein is packaged into virions, the degradative enzyme is
5 proteolytically cleaved from the fusion protein, i.e., it is soluble within the virion.

A method of inhibiting virus replication or infectivity is also provided by the present invention. A nucleic acid molecule encoding a fusion protein is introduced into a cell susceptible to a virus. The fusion protein may comprise a
10 capsid or envelope protein of the virus as well as a destructive enzyme as described above. The method of introducing the nucleic acid molecule into the cell is not critical to the invention, and many such methods are known in the art. These include, without limitation: infection, transformation, transfection, lipofection, tungsten microprojectiles, electroporation, cell fusion, and
15 transduction.

Nucleic acid molecules encoding the fusion protein of the present invention can be introduced into solid organs, tissues or cells, e.g., stem cells of the hematopoietic lineage or B lymphocytes. T-lymphocytes and monocytes/macrophages, which are derived from hematopoietic stem cells, are
20 the primary target of viruses such as HIV. Bone marrow cells can be taken from an individual, and if desired, the stem cells can be purified from the mixed population of marrow cells. The nucleic acid molecules of the present invention can be introduced into the stem cells by means of transfection, or any other means known in the art. The transfected stem cells can then be reinfused into the
25 individual (autologous stem cell transplantation). To facilitate proliferation of the transplanted stem cells, the individual's bone marrow can be partially cleared by irradiation or chemotherapy.

In another embodiment, the nucleic acid molecule of the present invention is introduced into an animal, or gametic cells or embryos. Techniques
30 such as microinjection or transfection can be used, as is well known to the art. The nucleic acid-treated cells can be used in artificial insemination (gametes) or can be reimplanted into a hormone-prepared female animal (embryos) as is known in the art.

In yet another method of introducing the nucleic acids of the present invention into cells or an animal, a recombinant virus can be used. The recombinant virus is desirably derived from a virus having the same cell-type tropism as the virus from which the capsid or envelope protein of the gene fusion is derived. Thus, for example, in order to introduce a gene fusion which employs a PERV capsid protein gene, a PERV viral vector may be used. The recombinant virus is then administered according to a route of viral infection for the particular virus of the vector.

An alternative way for introducing the fusion protein encoding construct of the present invention into cells and animals is by means of hybrid virus particles which contain the nucleic acid of the present invention but do not contain the fusion protein of the invention. Hybrid viruses can be prepared which contain the nucleic acid encoding the fusion protein of the present invention using a packaging cell line. Such cell lines may be propagated in culture according to techniques well known in the art. Such cell lines produce the proteins necessary for packaging viral genomes. The nucleic acids of the present invention can be introduced into such a packaging cell line. Desirably, the nucleic acids will contain the packaging signal which is recognized by the packaging proteins of the cell line. Preferably, the fusion protein gene is under the control of an inducible promoter, such as the metallothionine promoter. When it is desirable to produce viral particles carrying the nucleic acid of the present invention, the inducible promoter can be induced to turn on transcription of the fusion protein gene construct, and a protein synthesis inhibitor, such as cycloheximide, can be added so that the fusion protein transcript is not translated in the packaging cell line. Thus, the fusion protein gene construct is transcribed and packaged into the particles produced by the cell line, without interference from (co-assembly with) the fusion protein. When the packaged particles are used to infect cells, the nucleic acid encoding the fusion protein is expressed, and the fusion protein becomes co-assembled into the progeny particles, rendering them non-infectious.

Methods of producing animals, e.g., humans or other non-human animals, which are resistant to a virus are also provided by the present invention. A nucleic acid molecule which encodes a fusion protein as described above is

introduced into the germ line of an animal or an embryo, or to the animal, e.g., via DNA immunization or by infection with recombinant virus. Methods for the introduction of nucleic acid molecules to animals are known in the art (see, for example, Salter et al., 1991; Salter et al., 1989; Henninghausen, 1990; Ebert et al., 1991; Wright et al., 1991; Jaenish, 1988; Wagner et al. (U.S. Patent No. 4,873,191); Donahue et al., 1992; Rogers et al., 1993). All somatic cells resulting from development of zygotes containing the nucleic acid molecule are resistant to the virus from which the fusion protein capsid or envelope portion is derived. Methods for making transgenic animals which carry foreign genes in each cell of the animal are known in the art.

Receptor Interference (RI)

Another antiviral strategy targets the first step in the virus life cycle, the interaction of the viral envelope glycoprotein(s) with a host cell receptor. For example, retroviral infection of cells can be significantly reduced by expressing soluble forms of the host receptor, which bind the glycoproteins of the incoming virions before the virion can bind to the host receptor, or the viral envelope glycoproteins (the product of the *env* gene), which block the host receptors, directly preventing virion binding.

There are at least two distinguishable steps in viral entry; the first involves recognition and binding of the viral envelope glycoprotein to the host receptor, the second involves steps that lead to the fusion of the host and viral membranes. The *env* gene of retroviruses and lentiviruses encodes a polypeptide precursor that is subsequently processed into two glycoproteins, the surface glycoprotein (SU) which contains the domains that interact with the host receptor, and the transmembrane glycoprotein (TM) that anchors the SU protein to the membrane and appears to be directly involved with the fusion between the virus and cell membranes. Following membrane fusion, the viral core is released into the cytoplasm where the single-stranded RNA genome found in the virion is copied into double-stranded DNA (dsDNA) by the viral enzyme reverse transcriptase (RT). This reverse transcription process produces a linear dsDNA that is longer than the RNA genome from which it derives. The ends of the linear DNA contain terminally redundant sequences known as long terminal repeats (LTRs). Retroviruses, and vectors that derive from them, appear to

require the breakdown of the nuclear membrane that occurs during mitosis for the viral DNA to gain access to the cellular genome. Once this occurs, the linear viral DNA is inserted into the host genome by the virally encoded enzyme integrase (IN). The integrated viral DNA is called a provirus. Efficient

- 5 production of progeny viruses requires the integration of the viral genome into host DNA.

- Cells and animals engineered to express envelope glycoproteins have been shown to be highly resistant to that strain of retrovirus and to have less virus-associated pathogenesis (Federspiel et al., 1989; Nihrane et al., 1996; 10 Salter et al., 1989). Based on the receptor interference of endogenous ALV(E), Salter and Crittenden (1989) proposed that insertion of the ALV(A) envelope gene into the germline of chickens and its subsequent expression would provide resistance to infection by ALV(A). Their initial germline studies produced 23 lines of transgenic chickens using ALV-based retroviral vectors and proved two 15 major points. First, these studies showed that avian retroviruses can be artificially inserted into the germline of chickens at a reasonable efficiency, stably inherited by subsequent generations, and that the viral genes were expressed (Crittenden et al., 1989; Salter et al., 1987). Second, these studies showed that expression of the inserted subgroup (A) envelope glycoproteins 20 could inhibit subsequent ALV(A) infection by subgroup-specific receptor interference, both *in vitro* and *in vivo* (Salter et al., 1989; Salter et al., 1991).

- Two transgenic chicken lines, *alv6* and *alv11*, that did not produce infectious virus were extensively characterized at the RNA level in different tissues and at different stages of development. Viral RNA was expressed by both 25 lines in all tissues tested regardless of the stage of development; insert *alv6* expressed moderate levels of *env* RNA, while insert *alv11* expressed low levels of *gag* and *env*. Both lines, however, expressed much higher levels of viral RNA in the tissues associated with ALV pathogenesis, the bursa, thymus, and spleen. *In vitro*, both *alv6* (about 4400-fold) and *alv11* (about 11-fold) chicken embryo 30 fibroblasts (CEF) were resistant to ALV(A) infection. The susceptibilities of the *alv6* and *alv11* transgenic lines to ALV(A) infection and associated pathogenesis were compared to other properties (e.g., resistance measured *in vitro*, *env* expression levels, etc.) to determine which characteristics were important

indicators of an antiviral effect *in vivo*. None of the *alv6* birds were productively infected as determined by the absence of both infectious ALV(A) and ALV(A) neutralizing antibodies, while two-thirds of the *alv11* birds were productively infected by ALV(A). However, neither the *alv6* nor the *alv11* chickens showed significant ALV-induced pathogenesis. Thus, the level of Env glycoprotein expressed in the target tissues for ALV pathogenesis determined the magnitude of ALV interference (Federspiel et al., 1991).

Reticuloendotheliosis virus (REV) is very cytotoxic to avian cells *in vitro* presumably due to the fusionogenic properties of the envelope glycoproteins, especially the TM glycoprotein. REV stocks are therefore produced in D17 cells, a canine osteosarcoma line tolerant to REV infection. D17 cell lines expressing the REV envelope glycoproteins were significantly more resistant to REV infection (up to 25,000-fold) compared to controls. However, when the same REV envelope expression cassette was delivered into chicken embryo fibroblasts (CEF), the REV envelope gene sustained large deletions and the envelope proteins were not expressed due to cytotoxicity of the REV envelope glycoproteins to CEF cells. Thus, to reduce potential cytotoxic effects, it is preferred that soluble forms of viral envelope glycoprotein are employed. The soluble protein differs from the wild-type protein in that it lacks the transmembrane (TM) domain which anchors the protein in the membrane, and are therefore secreted from the cell.

The invention will be further described by the following examples.

Example 1

Expression of a Mo-MLV Gag-*E. coli* RNase H Fusion Polyprotein

A gene encoding a Mo-MLV Gag-*E. coli* RNase HI fusion was constructed by linking the RNase HI coding region to the Mo-MLV Gag polyprotein six codons upstream of the Gag termination codon (Van Brocklin et al., 1997). The RNase HI coding sequence was isolated from *E. coli* DNA by PCR amplification with a 5' primer containing a BglII site (5'-GCG CAT GCA GAT CTG ATG CTT AAA CAG GTA GAA ATT TTC ACC GAT GG-3'; SEQ ID NO:1) and a 3' primer containing a SalI site (5'-GCT GCT GCG TCG ACT TAA ACT TCA ACT TGG TAG CCT GTA TCT TCC-3'; SEQ ID NO:2). The reagents and conditions used for the PCR were as described in Federspiel et al.

(1996). The *E. coli* RNase H1 fragment was sequenced and contained one silent nucleotide difference from the sequence published by Kanaya and Crouch (1983), at nucleotide 470 (A→G). The Mo-MLV Gag-RNase H fusion construct was based on the design of the Mo-MLV Gag-SN fusion which has been used to introduce SN into virions (Natsoulis et al., 1995).

The genes encoding the CTVI constructs were delivered into CEF by the RCASBP(A) retroviral vector and expressed under the control of the viral promoter-enhancer in the long terminal repeat. CEF were cultured and passaged every 2 days when they reached confluence (Federspiel et al., 1994). ALV retroviral vector propagation was initiated by transfection of plasmid DNA that contained the retroviral vector in a proviral form by the calcium phosphate precipitation method (Kingston et al., 1989). The course of the retroviral infection was monitored by assaying culture supernatants from confluent cells for the viral Gag protein. Three plasmids were used for comparison: Mo-MLV Gag-SN plasmid pGN1600 (Natsoulis et al., 1995); Mo-MLV Gag plasmid pGN1601 (Natsoulis et al., 1995), which produces only the Mo-MLV Gag polyprotein; and Mo-MLV Gag-SN* plasmid pGS293 (Schumann et al., 1996), which differs from Mo-MLV Gag-SN in two missense mutations in the SN gene that result in an inactive SN enzyme. Maximum levels of Mo-MLV Gag, Mo-MLV Gag-RNase H, Mo-MLV Gag-SN, and Mo-MLV Gag-SN* were seen 10 to 14 days posttransfection, which coincided with maximum RCASBP(A) production. Immunoblot analysis indicated that the Mo-MLV Gag-RNase H polyprotein was expressed at a lower level than Mo-MLV Gag or Mo-MLV Gag-SN. Several viral proteins that were smaller than the expected full-length polyproteins were also observed. These proteins were the likely result of partial degradation of the Pr85 or Pr65 polyprotein associated with the virion isolation procedure, since smaller Gag-associated proteins were also seen in the Mo-MLV Gag control. Southern blot analysis of genomic DNA isolated from day 24 cultures detected only intact proviruses. An *in situ* RNase H assay showed that virus particles isolated from CEF expressing the Mo-MLV Gag-RNase H polyprotein contained high levels of RNase H activity. Little or no RNase H activity was detected in any other culture supernatants.

CEF Expressing the Mo-MLV Gag-RNase H Polyprotein Significantly Inhibit Mo-MLV Spread. The antiviral effect of the CTVI fusions expressed by the CEF cultures was measured first in a prophylaxis assay. The cultures were challenged with a low dose (multiplicity of infection [MOI] of 0.05 to 0.1 focus-forming units [FFU] per cell) of amphotropic Mo-MLV strain Mo(4070A) (Ott et al., 1990; Ott et al., 1992). Mo(4070A) is an engineered hybrid Mo-MLV that contains the *env* gene and a portion of the *pol* gene of the amphotropic 4070A virus. The Mo(4070A) virus stock was produced on NIH 3T3 cells (1×10^6 to 2×10^6 FFU/ml). Infectious virus was quantitated by the S+L- focus assay on D56 cells (Bassin et al., 1971). The CEF cultures, which were dividing rapidly, were passaged for 14 days after Mo(4070A) infection. Infectious Mo(4070A) was quantitated at day 14 by the S+L- focus assay (Table 1). CEF expressing the Mo-MLV Gag-RNase H polyprotein reduced the level of infectious Mo(4070A) produced by more than 1,500-fold compared to the Mo(4070A)-infected CEF control.

Table 1. Inhibition of Mo-MLV spread by CTVI^a

Construct used for treatment	Titer (FFU/ml) ^b (fold inhibition) ^c	
	Expt 1	Expt 2
None (mock treatment)	1.6×10^5	2.1×10^5
Vector alone	2.4×10^4 (6.6)	ND ^d
Mo-MLV Gag	2.5×10^4 (6.3)	4×10^4 (5)
Mo-MLV Gag-RNase H	1×10^2 (1,550)	9×10^1 (2,330)
Mo-MLV Gag-SN	5×10^1 (3,100)	1.6×10^1 (13,125)
Mo-MLV Gag-SN*	1.9×10^4 (8.4)	ND

^a In a prophylaxis assay, CEF cultures seven passages after transfection with plasmids containing the RCASBP(A) retroviral vector-CTVI constructs were challenged with Mo(4070A) (multiplicity of infection, 0.05 to 0.1 FFU per cell). Infectious Mo(4070A) levels were quantitated from supernatants 14 days postinfection by S+L- focus assays. This experiment was performed twice.

^b Titers were averages of duplicate assays.

^c Fold inhibition was determined by comparing the Mo(4070A) titers of the experimental constructs to the Mo(4070A) titer on mock-treated CEF.

^d ND, not done.

Several cultures were further characterized over the course of the 14-day Mo(4070A) infection by quantitating the levels of infectious Mo(4070A) by S+L- focus assay and analyzing the expression levels of the Mo-MLV Gag and RCASBP(A) Gag polyproteins by Western immunoblotting. The amount of infectious Mo(4070A) produced peaked 7 to 8 days after infection in the control cultures (CEF alone and CEF expressing Mo-MLV Gag), and the titer remained relatively constant over the next 6 days. In contrast, the amount of infectious Mo(4070A) produced by CEF expressing either the Mo-MLV Gag-RNase H or Mo-MLV Gag-SN polyprotein was significantly inhibited, although the levels of infectious virus slowly increased over the course of the experiment. Mo(4070A) virus production was also monitored by the appearance of the Mo-MLV CA protein (about 30 kDa) on immunoblots. The levels of expression of the Mo-MLV Gag, Mo-MLV Gag-RNase H, and Mo-MLV Gag-SN polyproteins remained relatively constant throughout the experiment. At late time points post-Mo(4070A) infection (days 12 to 14), the Mo-MLV Gag polyprotein levels may decrease slightly due to proteolytic processing by protease from the infectious Mo(4070A). The levels of the MA protein (about 19 kDa) from the RCASBP(A) retroviral vector remained relatively constant throughout the experiment.

The Mo-MLV Gag-SN fusion polyprotein was tested in parallel with the Mo-MLV Gag-RNase H fusion to provide a direct comparison to a characterized CTVI construct known to have antiviral activity. Unexpectedly, the antiviral effect of Mo-MLV Gag-SN was 100-fold greater in the experiments reported herein ($> 3,000$ -fold [Table 1]) than that previously observed (about 30-fold [Natsoulis et al., 1995]). The increase in the antiviral effect may be due to an increase in the efficiency of producing CEF cultures that express higher levels of the fusion polyprotein. The CEF themselves grew at a faster rate, presumably due to changes in the growth media including different serum lots. This difference in growth rate was especially apparent in CEF infected with Mo(4070A). In previous experiments, Mo(4070A) infection dramatically slowed the growth of CEF and caused some cytotoxicity.

In the experiments reported herein, all CEF cultures grew at the same rate until natural senescence. A consistent six- to eight-fold inhibition of Mo(4070A)

titer was observed in cells expressing the control constructs, including cultures that expressed RCASBP(A) vector (Table 1), that was not detected previously (Natsoulis et al., 1995; Schuman et al., 1996). Although this may be related to the "steric effect" (three- to five-fold) of the Mo-MLV Gag-SN* polypeptide on Mo(4070A) virus production observed by Schumann et al. (1996), the decrease in the Mo(4070A) titers produced in cultures that expressed RCASBP(A) suggests that the RCASBP vector may alter cells in ways that interfere with Mo-MLV virus production, leading to a slight overestimate of the CTVI effect. While several CTVI constructs have significant antiviral effects when used prophylactically, the levels of infectious virus do increase during the experiment. The production of a small number of viruses that do not contain CTVI-derived polypeptides most likely accounts for the small number of infectious viruses needed to spread the infection. Mutations in the *gag* gene capable of circumventing this antiviral strategy would likely be exceedingly rare since they would require the selection of Gag mutants that would still assemble normally but be able to exclude Gag-degradative enzyme polypeptides from virions.

Delivery of the Mo-MLV Gag-RNase H Fusion Polypeptide to Mo-MLV-Infected CEF has a Therapeutic Effect. To assess the therapeutic potential of the CTVI strategy, CEF chronically infected with Mo(4070A) were transfected with the RCASBP(A) retroviral vector plasmids containing the Mo-MLV Gag fusions and passaged for 16 days. For the therapy assay, CEF chronically infected with Mo-MLV were generated by infecting 4×10^6 cells with 2×10^6 FFU of Mo(4070A) (1 ml of virus stock) and passaging the culture four to six times to allow maximum spread of the virus. The Mo(4070A)-infected CEF cultures produced a maximum titer of 3×10^5 to 4×10^5 FFU/ml as quantitated by the S+L- focus assay. The RCASBP virus spread through Mo(4070A)-infected CEFs with kinetics similar to the spread on CEF that had not been infected with Mo(4070A), reaching maximum levels after about 8 days as determined by Western analysis of ALV MA levels. The levels of infectious Mo(4070A) were quantitated throughout the experiment. The Mo(4070A) titers at 14 days posttransfection in two experiments are shown in Table 2. The level of infectious Mo(4070A) was reduced 7.5- to 18-fold in CEF expressing the Mo-MLV Gag-RNase H polypeptide and 15- to 38-fold in CEF expressing the Mo-

MLV Gag-SN polyprotein. Infection with the RCASBP(A) vector alone or RCASBP(A) expressing the Mo-MLV Gag polyprotein lowered the titer of Mo(4070A) produced by a chronically infected culture two- to three-fold compared to Mo-MLV-infected CEF. This suggests that only a slight

- 5 therapeutic effect is directly attributable to the CTVI constructs once the infection is established.

Table 2. Inhibition of Infectious Mo-MLV Produced after CTVI Treatment^a

Construct used for treatment	Titer (FFU/ml) ^b (fold inhibition) ^c	
	Expt 1	Expt 2
None (mock treatment)	3×10^5	3.8×10^5
Vector alone	1.5×10^5 (2)	ND ^d
Mo-MLV Gag	1×10^5 (3)	1.6×10^5 (2.4)
Mo-MLV Gag-RNase H	4×10^4 (7.5)	2.1×10^4 (18)
15 Mo-MLV Gag-SN	2×10^4 (15)	1.3×10^4 (29)

^a In a therapy assay, CEF chronically infected with Mo(4070A) were transfected with plasmids containing the RCASBP(A) retroviral vector-CTVI constructs. Levels of infectious Mo-MLV in supernatants were quantitated 14 days posttransfection by S+L- focus assays. The experiment was performed twice.

^b Titers were averages of duplicate assays.

^c Fold inhibition was determined by comparing the mo-MLV tiers of the experimental constructs to the Mo-MLV titer on mock-treated CEF.

^d ND, not done.

Cleavage of the Mo-MLV Gag-Nuclease Fusion Junction by the

Incoming Mo-MLV Protease. An Mo-MLV Gag polyprotein fusion junction

30 was designed that could be cleaved by the protease of an incoming infectious Mo-MLV by including the region six amino acids on either side of the normal Mo-MLV protease cleavage site (PRCS). The new Gag fusion plasmid moves the BamHI site to a position just downstream of the Gag termination codon (pMGagPRCS). The termination codon was changed to a codon for glutamine,

35 the same amino acid that Mo-MLV inserts during suppression of the stop codon when the Gag-Pol polyprotein is produced. Genes encoding the Mo-MLV Gag PRCS-RNase H and Mo-MLV Gag PRCS-SN polyproteins were prepared to determine if cleavage of the RNase HI or SN domain from the polyprotein would

- increase the antiviral effect. A prophylaxis assay was used to measure Mo(4070A) production in cells expressing the Mo-MLV Gag PRCS fusions and the original Mo-MLV Gag fusions. Addition of the PRCS did not alter the level of inhibition by the RNase HI or SN polyprotein, nor did inclusion of the PRCS
- 5 alter the therapeutic antiviral effect of either the Mo-MLV Gag-RNase H or the SN polyprotein. Virion proteins isolated from day 16 of the therapy assay of Mo-MLV-infected CEF and CEF expressing Mo-MLV Gag, Mo-MLV Gag-RNase H, and Mo-MLV Gag PRCS-RNase H were assayed for RNase H activity by *in situ* RNase H assay. Only virions from Mo-MLV Gag-RNase H and Mo-
- 10 MLV Gag PRCS-RNase H cultures contained measurable RNase H activity. A new protein with RNase H activity about 22 kDa was observed in virions obtained from cultures expressing the Mo-MLV Gag PRCS-RNase H polyprotein. Presumably, this protein, which corresponds in size to *E. coli* RNase HI, is the RNase HI domain cleaved from the Mo-MLV Gag PRCS-
- 15 RNase H polyprotein by the Mo-MLV protease. The ability to release a degradative enzyme from the polyprotein should make it possible to design

future CTVI fusions in which activation of the degradative enzyme is controlled by protease cleavage (e.g., a zymogen), which would allow the use of degradative enzymes whose expression would otherwise be too toxic for the cell.

CTVI Inhibition of Mo-MLV Replication in Avian DF-1 Cells. Line 0

- 5 CEF have been the cells of choice for the study of ALV, since line 0 does not contain endogenous loci related to ALV and produces high ALV titers. However, CEF are primary cells and have a limited life span (i.e., about 25 passages). Recently, a permanent cell line was derived from line 0 CEF, named DF-1 (Dr. Douglas Foster, University of Minnesota). Previously, permanent
- 10 avian cell lines have been unsatisfactory for many ALV studies due to the poor growth of ALV or the presence of interfering exogenous and/or endogenous viruses. The growth of ALV-based retroviral vectors and Mo-MLV was compared in DF-1 and CEF. The DF-1 and CEF cells grow at similar rates and support vigorous growth of ALV and Mo-MLV. The rate at which both ALV
- 15 and Mo-MLV reach maximum titers, and the level of viral protein produced, is approximately the same in either cell type. The titer of the ALV-based retroviral vectors is 5-10-fold higher when produced in DF-1 cells compared to CEF. A slight increase in the titer of Mo(4070A) viral stocks was obtained when grown in DF-1 cells compared to CEF. These data demonstrate that the DF-1
- 20 permanent cell line can be used to test antiviral strategies *in vitro*, and also to generate clonal cell lines that support vigorous ALV replication. Table 3 summarizes the CTVI antiviral effects on Mo-MLV replication in DF-1 cells both prophylaxis and therapy assays.

- In animal model systems, it is possible to deliver genes by using either
- 25 DNA microinjection or retroviral infection into an embryo. This eliminates the host's immune response, and such methods can be used to deliver genes efficiently. Thus, the CTVI strategy may be useful *in vivo*, e.g., in animal systems in which viral infections have important economic consequences (e.g., agriculture), or in non-human transplant tissues.

Table 3. CTVI inhibition of Mo-MLV replication in avian DF-1 cells

	Construct	Titer (FFU/ml) ^a (fold inhibition) ^b	
		Prophylaxis	Therapy Assay ^d
		Assay ^c	
5			
			1st Delivery ^e 2nd Delivery ^f
	None (mock)	3×10^5	2×10^5 2.1×10^5
	Mo-MLV Gag	1.2×10^5 (2.5)	1.4×10^5 (1.4) 1.4×10^5 (1.5)
10	Mo-MLV Gag-RH	<10 (>30,000)	1×10^2 (2,000) <10 (>21,000)
	Mo-MLV Gag-SN	<10 (>30,000)	1×10^2 (2,000) <10 (>21,000)

^a Titers were averages of duplicate assays.

^b Fold inhibition was determined by comparing the Mo(4070A) titers of the construct to mock-treated DF-1 cells.

^c DF-1 cells chronically infected with RCASBP(A)-CTVI construct were challenged with Mo(4070A) (multiplicity of infection, 0.05-0.1 FFU per cell). Infectious Mo(4070A) levels were quantitated from supernatants by S+L- assay 30 days post-infection.

^d DF-1 cells chronically infected with Mo(4070A) were infected with RCASBP(A)-CTVI or RCASBP(C)-CTVI construct viruses. The levels of infectious Mo(4070A) in supernatants were quantitated by S+L- assay 30 days post-infection.

^e RCASBP(A)-CTVI viruses were used to initiate the therapy assay with the maximum antiviral effect achieved at 30 days.

^f The antiviral constructs were delivered a second time by the RCASBP(C) vectors.

Example 2

Human Tropic PERVs Produced by Pig Primary PBMC

Methods

- Cells and Cell Culture.** The cell lines used were either obtained from the ATCC unless otherwise indicated. 293 human embryonic kidney (ATCC CRL-1573), MMK *Mus musculus molossinus* kidney (ATCC CRL-6439), SC-1 mouse embryo epithelial cells (ATCC CRL-6450), ST-IOWA (pig fetal testis), 1o PT (pig testis), MDTF *Mus dunni* tail fibroblast (kindly provided by Olivier Danos), NRK normal rat kidney cells (ATCC CRL-6509), Rat2 rat embryo fibroblasts (ATCC CRL-1764), SIRC rabbit corneal fibroblast (ATCC CCL-60), D17

- canine osteosarcoma (ATCC CRL-6248), MDBK bovine kidney epithelium (ATCC CCL-22), FRHK4 rhesus monkey kidney cells (ATCC CRL-1688), Mv1Lu mink lung epithelial cells (ATCC CCL-64), MiCL.1 (S+L-) mink lung (ATCC CCL-64.1), Fe3TG feline tongue fibroblast (ATCC CCL 176), AK-D feline lung epithelial cells (ATCC CCL-150), CRFK feline kidney epithelial cells (ATCC CCL-94), CaKi-1 clear cell kidney carcinoma (ATCC HTB-46), HeLa cervical adenocarcinoma (ATCC CCL-2), HOS human osteosarcoma (ATCC CRL-1543), CaCo-2 colorectal adenocarcinoma (ATCC HTB-37) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 1 mM sodium pyruvate, 100 U of penicillin per ml, and 100 µg of streptomycin per ml. PG-4 feline glial and astrocytes (ATCC CRL 2032) were maintained in McCoy's 5A medium supplemented with 15% FBS, 2 mM glutamine, 1 mM sodium pyruvate, 100 U of penicillin per ml, and 100 µg of streptomycin per ml. HepG-2 hepatoblastoma (ATCC HB-8065) and HT1080 fibrosarcoma (ATCC CCL-121) were maintained in Eagle's Modified Essential Medium supplemented in a similar manner. The following cell lines were maintained in RPMI 1640 supplemented with 10% FBS, 2 mM glutamine, 0.05 mg gentamicin sulfate per ml, 1X non-essential amino acids (Biofluids, Rockville, MD): Molt 4 acute lymphoblastic (ATCC CRL-1582), Daudi Burkitt's lymphoma (ATCC CCL-213), Raji Burkitt's lymphoma (ATCC CCL-86), U937 histiocytic lymphoma (ATCC CRL 1593.2) UCLA-SO-M14 T cells (Ramsdell et al., 1988), and the human natural killer cell line YTN10 (Yodoi et al., 1985). PBMCs were obtained from NIH and Yucatan minipigs.
- Primary pig aortic endothelial cells are isolated by incubating the inner layer of pig aortas with 0.2% collagenase type I for 20 minutes at 37°C. Detached cells are washed with phosphate-buffered saline, and cultured in M199 medium supplemented with 10% fetal bovine serum, 25 mM HEPES, 2 mM glutamine, 100 U per ml of penicillin, and 100 µg streptomycin. Cultures are fed three times per week and subpassaged every 4-7 days.
- Pig hepatocytes are isolated by a two-step perfusion technique as described by Sielaff et al. (1995). The liver is treated by systemic heparinization before isolation. The liver is then treated with an EDTA and collagenase D

solution to free the hepatocytes. The hepatocytes are isolated by gently combing the liver, washing the dislodged cells in Williams E medium supplemented with 5% calf serum, 2 mM L-glutamine, 15 mM HEPES, 1.5 mg/ml insulin, 100 U penicillin per ml and 100 µg streptomycin per ml. The freshly harvested

5 hepatocytes are cultured in collagen gel.

Derivation of viral pseudotypes. Cells productively infected with wild-type PERV were superinfected with retroviral vector-containing supernatant harvested from PA317/G1BgSvN (McLaughlin et al., 1993), in a manner similar to that previously described (Leverett et al., 1998), to generate PERV

10 pseudotypes carrying the murine retrovirus genome coated by PERV core and envelope proteins. G1BgSvN-containing cells were selected in 187 µg/ml G418 (active component). After 10-14 days selection, G418-resistant colonies were pooled and used to generate viral pseudotypes. Since the G1BgSvN vector genome contains the coding sequence for both neomycin phosphotransferase and
15 for β-galactosidase, pseudotype infection is monitored by the acquisition of target cell resistance to G418 or by immunohistochemical detection of target cells expressing β-galactosidase.

Infectivity assays. Supernatant infections were used to determine the infectivity of cell-free virus derived from different producer cell lines.

20 Comparisons between fresh and frozen virus-containing supernatants demonstrated that storage of viral supernatants at -70°C resulted in > 100-fold decrease in infectious titer. Therefore, all experiments are performed using freshly harvested supernatant. On the day prior to initiating an infectivity assay, target cells were seeded at $3-5 \times 10^4$ cells per well in 12-well dishes. On the next
25 day, supernatant from post-confluent cultures of virus producer cells was harvested, filtered through a 0.45 µm filter, adjusted to 6 µg/ml polybrene and used to replace the medium on target cells. On the following day, the cells were fed with fresh medium. When β-galactosidase expression was used to monitor infectivity, the cells were fixed and histochemically stained 48-72 hours post-
30 exposure to viral supernatant and observed microscopically to enumerate blue-staining foci of cells (BFU/m1), as described elsewhere (Wilson et al., 1991). If the experiment was performed to assess productive infection of the target cell,

the cells would be passaged 1-2 times per week as needed during the course of the experiment and monitored as described below.

The coculture of target cells with virus-producer cells was used to assess the ability of the virus to be transmitted to non-adherent cells. Since several different cell types were used as a source of virus-producer cells in these experiments, the dosage of irradiation required to ensure the virus-producer cells would die within 5 days after irradiation was first determined. Three to four x 10⁶ cells for each virus-producer cell line were subjected to 2,000, 5,000 or 10,000 rads (using a ¹³⁷Cs source, Nordion GammaCell 100) and seeded into a 96-well plate at 2-3 x 10⁵ cells/well. Irradiated and control non-irradiated cells were monitored for proliferation daily for 5 days by measuring [³H]TTP uptake after cells were cultured for 8 h with 1.0 µCi [³H]TTP (6.7 Ci/mmol; Dupont NEN, Boston, MA), harvested (Skatron, Sterling, VA) and processed for scintillation counting. Based on these findings, the following conditions were used: Daudi cells received 2000 rad, Molt4 and U937 received 5000 rads.

When the target cells in the coculture were human peripheral blood mononuclear cells (hPBMC), the mononuclear layer was collected from a buffy coat from a human donor separated by lymphocyte separation medium (Organon Teknika, Durham, NC). Cells were stimulated in 1X PHA (according to manufacturer's instructions, Life Technologies, Gaithersburg, MD) for 3 days and then mixed in a 1:1 ratio with 3 x 10⁶ lethally irradiated virus-producer cells in 12-well dishes. Thereafter, the cocultures were maintained in RPMI 1640 medium supplemented with 10% FBS, 10% purified human IL-2 (Boehringer Mannheim), 10 ng/ml recombinant human IL-2 (Chiron Corporation, Emeryville, CA) 2 mM glutamine, 100 U penicillin, and 100 µg per ml of streptomycin. Control wells containing irradiated virus-producer cells only were monitored for cell viability by visualizing samples microscopically for trypan blue exclusion. Cocultures containing 293 cells as target cells were carried out in parallel as a control for the infectivity of the virus-producer cells. After initiation of the coculture, the next day the medium was replaced with fresh medium and cells were sub-cultured 1-2 times/week as needed. Cocultures containing the hPBMC as targets were reinfused every two weeks with freshly-stimulated hPBMC from the same buffy coat of cells. Methods to monitor virus infection of target cells.

Infection of target cells was assessed by one of three different methods. In some cases, the titer of infectious virus pseudotypes was determined by β -galactosidase expression. Cells were histochemically stained 48-72 hours after exposure to virus-containing supernatant, and foci of β -galactosidase expressing blue cells were counted (BFU/ml). Those titers were then normalized to the titer observed on 293 cells exposed to the same viral supernatant and are reported as % of control. The titer observed on 293 cells ranged 10-fold from several hundred BFU/ml to 2-3,000 BFU/ml.

Virion isolation and purification. Culture supernatants are harvested from infected cells, cleared of floating cells and debris by centrifugation at 3000 $\times g$ for 10 minutes, and the virus stocks stored at -80°C . Virions are isolated for RT assays and Western analysis by ultracentrifugation of 10 ml virus stock underlaid with 1 ml 20% sucrose at 35,000 rpm in a Beckman SW40 rotor for 1 hour at 4°C to pellet the virus (Federspiel et al., 1994). The viral pellet is then resuspended in either RT assay buffer or Laemmli load buffer.

PERV virions are further purified for antibody production either by sucrose equilibrium density centrifugation or by a sucrose step gradient to concentrate the virions and remove proteins, nucleic acids and ribosomes from the cell supernatants. For equilibrium gradients, the supernatants are mixed with sucrose to a density slightly higher than 1.16 g/ml, the average buoyant density of retroviral particles, and the gradient formed by ultracentrifugation. Viral supernatants are layered onto a two-step sucrose gradient, 20% and 50%, and the virions banded at the boundary between the sucrose steps. In both methods, the banded virions are harvested by removing the band with a syringe through the side of the tube to prevent contamination.

PCR. Standard PCR reactions contains 1.25 μl of 10X PCR buffer (50 mM Tris, pH 8.3, 50 mM KCl, 7 mM MgCl_2), 1.25 μl of 1.7 mg/ml BSA, 0.5 μl of each dNTP at 25 mM, 0.56 μl of each oligonucleotide primer ($A_{260} = 5$), 0-8% dimethyl sulfoxide, 4.2 μl water, and 1 μl genomic DNA (about 100 $\mu\text{g}/\text{ml}$) (Federspiel et al., 1996). The reactions are heated to 90°C and initiated with 1.5 μl *Taq* DNA polymerase (0.75 units). Thirty cycles of PCR are carried out as follows: 90°C for 20 seconds, then 59°C for 80 seconds. Alternations of this basic protocol follow the recommendations of the polymerase manufacturer.

- Three sets of primers specifically amplify the three PERV envelope subgroups in the SU glycoprotein region: (all primers listed 5'-3') PERV-A: TGGAAAGATTGGCAACAGCG and AGTGATGTTAGGCTCAGTGG (SEQ ID NO:8 and SEQ ID NO:9, respectively); PERV-B:
- 5 TTCTCCTTTGTCAATTCCGG and TACTTTATCGGGTCCCACTG (SEQ ID NO:10 and SEQ ID NO:11, respectively); PERV-C: CTGACCTGGATTAGAACTGG and ATGTTAGAGGATGGTCTCTGG (SEQ ID NO:12 and SEQ ID NO:13, respectively). PCR products were separated on a 1.0% agarose gel and DNA fragments of 2-2.5 k B were purified using Qiaex gel
- 10 purification kit (Qiagen, Valencia, CA). Purified PCR products were then cloned into the TA vector using the TA Cloning Kit (In Vitrogen, Carlsbad, CA). Restriction analysis of clones derived from the TA vector was used to ensure an insert of the appropriate size was present. A minimum of 4 representative clones was chosen for sequence analysis. All deoxynucleotide sequencing was
- 15 performed by the Mayo Clinic Molecular Biology Core on a Perkin Elmer ABI PRISMTM 377 DNA Sequencer (with XL Upgrade) with the PE applied Biosystems ABI PRISMTM dRhodamine Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase (PE Applied Biosystems, Foster City, CA). The ClustalW Multiple Sequence alignment Program of Mac Vector
- 20 6.0.1 (Oxford molecular Group) was used to compare the deduced envelope amino acid sequences.

- RT-PCR.** RNA is isolated from virions with RNA STAT 50-LS or STAT-60 (Tel-Test, Inc., Friendswood, TX), and converted to cDNA with 2.5 μM random hexanucleotide primers and Superscript II (Life Technologies,
- 25 Gaithersburg, MD) (Wilson et al., 1998). cDNA templates are amplified with primers corresponding to the PERV *pol/env* gene boundary (ACCTCGAGACTCGGTGGAAGGG; SEQ ID NO:14) and the untranslated region 3' of the PERV *env* gene (CTGGGTCTGGGAGGGTTAGGTTG; SEQ ID NO:15), or amplified with PB906 (5'
- 30 ACGTACTGGAGGAGGGTCACCTGA 3'; SEQ ID NO:16) and PB908 (5' GTCCCGAACCCTTATAACCTCTTG 3'; SEQ ID NO:17) or PERVenv1 (sense) (5' ACCTCGAGACTCGGTGGAG; SEQ ID NO:11) and PERVenv2 (anti-sense) (5' CTGGGTCTGGGAGGGTTAGGTTG; SEQ ID NO:12) for

30 cycles at 94°C for 30 seconds, at 60°C for 30 seconds, and at 72°C for 1 minute. The amplified products are cloned into the PCR II T-A vector (Invitrogen Corp., San Diego, CA).

- RT assays. The RT assays are conducted as described in Wilson et al. (1994) and Wilson et al. (1998) and are optimized for the detection of PERV RT as reported by Phan-Thanh et al. (1992). Pelleted virions or virions in cleared supernatant are treated in solubilization buffer (25 mM NaCl, 0.20% Triton X-100, 10 mM Tris, pH 7.5) for 15 minutes at ambient temperature. The solubilized samples are incubated for 3 hours at 37°C in 2X substrate buffer (50 mM Tris, pH 7.5, 10 mM dithiothreitol, 0.6 mM MnCl₂, 10 µg of poly (rA)-poly (dT)₁₂₋₁₈ (Pharmacia, Piscataway, NJ) per ml, and 10 µCi of ³H[TTP] per 25 µl (22 Ci/mmol). The incubation is terminated with EDTA followed by blotting the synthesized DNA on a DEAE filtermat (LKB-Wallac, Gaithersburg, MD) through use of a harvesting apparatus (Skatron, Sterling, VA) with a rinse of 1% Na₂HPO₄ (pH 7.0) containing 1 mM EDTA. Radioactivity is then determined via liquid scintillation. All samples are assayed in triplicate.

- RNAse protection assays (RPA). RNA from viral particles or cells is extracted using RNAzol™ B (Tel-Test, Inc., Friendswood, TX). RPA are done with the RPA II Ribonuclease Protection Assay Kit (Ambion, Inc., Austin, TX). Antisense riboprobes are synthesized in the presence ³²P[UTP] using the RNA Transcription Kit (Stratagene, La Jolla, CA). In standard RPA conditions, labeled riboprobe (5 × 10⁵ cpm) is incubated with the RNA at 50°C overnight. The annealed reaction is digested with an RNase A/T1 mix at 30°C for 45 minutes. The samples are cleaned up and fractionated on 6% acrylamide-7.6 M urea gels, dried and exposed to Kodak X-OMAT film.

- Western analysis. Virion proteins were isolated and separated on 12% SDS polyacrylamide gels. The proteins were transferred to nitrocellulose filters with an Enprotech blotting apparatus (Integrated Separation Systems, Hyde Park, MA). The filters were blocked in phosphate-buffered saline with 10% nonfat dry milk (wt/vol) for 1 hour at ambient temperature. The blocked filters were drained and incubated with a 1:1000 of goat anti-GALV CA or goat anti-SSAV CA antisera in rinse buffer (100 mM NaCl, 10 mM Tris, pH 8, 1 mM EDTA, 0.1% Tween 20) containing 1% nonfat dry milk for 1 hour at ambient

temperature (Biological Carcinogenesis Branch Repository of the National Cancer Institute). The filters were then washed extensively in rinse buffer, and incubated with 50 ng/ml peroxidase-labeled rabbit anti-goat IgG (H + L)

(Kirkegaard and Perry, Gaithersburg, MD) in rinse buffer with 1% nonfat dry

- 5 milk for 1 hour at ambient temperature. The filters were washed and the protein-antibody-peroxidase complexes detected with the Western Blot Chemiluminescence Reagent (DuPont NEN, Boston, MA). The filters were exposed to Kodak X-OMAT film.

Results

10 Host Range of PERV Isolates

Primary PBMC were isolated from an NIH miniature pig and exposed to two different combinations of mitogens: phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) or PMA and the calcium ionophore A23187.

Mitogenic activation by either treatment resulted in a sharp increase in reverse

- 15 transcriptase (RT) activity levels five days after stimulation (Figure 1A). To determine if the increase in RT activity correlated with the production of infectious virus, stimulated PBMC from NIH and Yucatan minipigs were cocultured with the pig ST-IOWA cell line, a line that does not produce significant endogenous RT activity levels in the supernatants but is susceptible to

- 20 PERV infection. Both cultures produced detectable RT activity 14 days after exposure to activated PBMC (Figure 1B). These results indicated that mitogenic activation of the pig PMBC released PERV capable of productive infection of pig cells. To determine if the produced PERV can infect human cells, stimulated PBMCs were cocultured with the human embryonic kidney 293 cell line. RT
25 activity was detected in 293 cells at day 31 (Figure 1C) and continued to increase to levels comparable to RT activity levels produced by infected ST-IOWA cells. These results clearly demonstrated that the stimulated pig PBMC produce PERV that can productively infect human cells. These findings suggest that the stimulation of porcine lymphocytes, transferred to the patient in a xenograft, may
30 result in the production of replication-competent human-tropic PERV capable of infecting the patient.

In order to test if the PERVs of this primary 293 infection (1°-293/PERV) could improve their infection efficiency in human cells, supernatant

from the 1°-293/PERV culture was passaged into a new culture of human 293 cells (2°-293/PERV) and allowed to spread through the culture (Figure 2). After the culture reached maximum RT activity, supernatant from the 2°-293/PERV culture was passaged into fresh 293 cells and allowed to spread (3°-293/PERV).

- 5 The titer of the PERVs produced by these three cultures was quantitated by generating MLV- β gal pseudotypes.

The titers of all three PERV cultures were approximately the same on the control ST-IOWA cells (Figure 3). However, the titers of the MLV- β gal/PERV pseudotypes produced from the 2°-293 and 3°-293 cultures were 5-fold higher
10 compared to the 1°-293 culture. The relative copy number of integrated PERV proviruses in these three PERV infected 293 cultures was quantitated by Southern analysis. As shown in Figure 4, the DNA isolated from the 2°-293/PERV and 3°-293/PERV cultures contained more integrated PERV proviruses per cell relative to the DNA of the 1°-293/PERV cultures.

- 15 Overall, the host range of the PERVs produced from the 293/PERV cultures appears to coincide with a PERV-A related isolate (see Table 4 compared to Takeuchi et al., 1998). One notable exception is the inability of PERV-1.15 MLV- β gal pseudotypes to infect D17 cells, suggesting that the changes in the envelope glycoproteins may have altered the host range.

Table 4. Host range of PBMC PERV produced by 293 cells

Species	Cell	Titer of <i>LacZ</i> pseudotype ^a		
		1°	2°	3°
Human	HeLa	+	+	+
	HOS	+	+	+
	Caki-1	-	-	+
	293	+	++	++
Primate	FRhK-4	-	-	-
Pig	ST-IOWA	++	++	++
	1o PT	+	+	+
Dog	D17	-	-	-
Rabbit	SIRC	-	-	-
Hamster	E36	-	-	-
Mink	Mv-1-Lu	++	++	++
	MiCl1	+	++	++
Cat	Fc3Tg	+	+	++
	AK-D	+	+	++
	CRFK	++	++	++
	PG4	++	++	++

^a MLV-βgal pseudotypes were produced in the PERV infected human 293 cultures: negative (-); 1-100 BFU/ml (+); >100 BFU/ml (++).

30

Table 5. Productive infection of human and animal cell lines by 293/PERV

Cell Line	Virus ^a	LacZ ^b	RT ^c	RT-PCR ^d
5	ST (pig testis)	1°	++	neg
		2°	++	d.9
		3°	++	d.9
	293 (human kidney)	1°	+	d.18
		2°	++	d.10
		3°	++	d.10
10	Caco-2 (human colon)	1°	?	neg
		2°	?	neg
		3°	?	d.18
15	HepG2 (human liver)	1°	++	neg
		2°	++	d.26
		3°	++	d.26
	HT1080 (human fibrosarcoma)	1°	+	neg
		2°	++	d.26
		3°	++	d.13
20	Mv-1-Lu (mink lung)	1°	+	neg
		2°	++	d.21
		3°	++	d.38
	CRFK (cat kidney)	1°	+	neg
		2°	+	d.14
		3°	++	d.14

25 ^a Culture supernatant from 1°, 2°, or 3° - 293/PERV cells.

^b Titer of MLV-βgal pseudotype: negative (-); 1-100 BFU/ml (+); >100 BFU/ml (++)

^c The day when the RT activity was greater than 2-fold over background activity from uninfected cells. The assay was terminated after 8 weeks.

30 ^d At 8 weeks postinfection, RNA was isolated from the culture supernatant and assayed for *pol* sequences by RT-PCR.

nd Not determined.

? High background staining made the data uninterpretable.

35

PERV Produced by the 293/PERV Cultures Can Productively Infect

Other Human, Mink and Cat Cell Lines. A number of different cell lines were employed to determine the tissue and species specificity of PERV produced by 293/PERV cultures (Table 5). All the cell lines tested except Caco-2 were productively infected by the virus populations produced by the 2° and 3° - 293/PERV cultures. The 3° - 293/PERV infected Caco-2 culture produced RT

activity at day 18 and detectable *pol* RNA by RT-PCR indicating a productive infection. The 1°-293/PERV culture produces 5-fold lower PERV titers (see Figure 3) which may account for the lower replication efficiency in most lines. The transfer of significant levels of replication-defective PERV genomes into the primary 293 cells may account for the lower titers. The ability of the PERV produced by the 2° and 3°-293/PERV cultures to efficiently replicate in most cultures may be the result of the loss of defective interfering PERV genomes or the adaptation of the virus, or both.

An expanded analysis of species tropism indicated that cell lines derived from mouse, rat, rabbit, dog, cow, and rhesus monkey were resistant to infection by 293/2° pseudotypes, while cell lines from cat or mink cell lines were susceptible. This latter susceptibility was low compared to 293 cells, with the exception of MiCL and PG4 cell lines. Mink and cat cell lines were also infected with PERV infected 293/2° supernatant. Only low levels of RT activity were observed in MiCL and CRFK cultures. PG-4 cultures had RT activity of 933 cpm [³H-TTP] at 3 weeks, which was reduced to 2344 cpm by 5 weeks, post infection.

Analysis of the Susceptibility of Hematopoietic Cells to Infection by PERV. In order to assess whether primary human PBMC (hPBMC) were susceptible to infection by PERV, PHA-activated hPBMC were cocultured with irradiated 293/2° virus-producer cells and maintained in IL-2-containing medium for 8 weeks. No RT activity above background levels or viral RNA as measured by RT-PCR were produced during the course of the experiment. Since maintenance of the hPBMC in IL-2 during the course of the experiment biases the culture conditions towards the proliferation of T cells, other hematopoietic lineages that may be susceptible to infection may not have been represented. To investigate the possibility that other hematopoietic lineages may be permissive for infection, a number of human hematopoietic cell lines representing the T cell, B cell, myeloid, and NK cell lineages were analyzed for their susceptibility to PERV infection by coculture with irradiated 293/2° virus producer cells. Supernatants from the cocultures containing either M14 (T cell lineage), Jurkat (T cell lineage), K562 (myeloid lineage), or the NK cell line YTN10, remained negative for RT activity during the 8 week course of the experiment, although

viral RNA could be detected by RT-PCR throughout the experiment. Figure 5 shows the data from the RT activity for the cocultures containing the T cell line Molt 4, the B cell lines Daudi and Raji, and the myeloid cell line U937. By 4 weeks post-coculture, the RT activity in the Molt 4- and Daudi-containing cocultures was positive and continued progressively to increase throughout the period of the coculture. In contrast, the RT activity for both the Raji and U937 sets of cocultures plateaued by 4 weeks post-culture. The results from this experiment suggest that the Molt 4, Daudi, Raji, and U937 cell lines were permissive for productive infection by PERV.

The RT positive hematopoietic cell lines were then co-cultured with primary hPBMC, as a virus population may have been selected in the susceptible hematopoietic cell lines that could more efficiently infect primary hematopoietic cells. The irradiation conditions optimal for lethal irradiation were determined for each of the RT positive Daudi, Molt 4 and U937 cell lines. Then lethally-irradiated RT-positive Daudi, Molt 4 and U937 were each employed as virus-producer cells in cocultures with primary hPBMC activated with PHA or with human 293 cells as positive controls. By 2 weeks post-coculture, each of the cocultures containing 293 cells as target cells became significantly RT positive (>7,000 cpm), and by 3 weeks, the RT activity in each of these cultures increased to >20,000 cpm. By contrast, none of the cocultures containing the hPBMC demonstrated RT activity higher than negative control cultures over the course of the experiment. Although viral RNA was detected by RT-PCR in the supernatant of cultures sampled 1-2 weeks post-exposure, the supernatant was negative for viral RNA by RT-PCR by three weeks and remained so out to the 8-week time point. The positive RT-PCR results obtained at the early time points most likely reflect presence of residual irradiated virus-producer cells which disappear from the culture at the later time points, rather than infected cells.

Table 6. Comparison of the nucleotide and deduced amino acid sequence of PERV-1.15 with the three known PERV *env* subgroups.

5

	1.15	A	B	C	Amino Acid Identity (%)
PERV-1.15	-	96	85	73	
PERV-A	97	-	82	76	
PERV-B	87	84	-	70	
PERV-C	72	76	72	-	
Nucleotide Identity (%)					

10

Env Subgroups of PERV in PERV- infected 293 Cultures

To determine what PERV envelope subgroups were present in virions produced by the 293/PERV populations, the envelope regions was cloned by RT-PCR amplification of virion RNA isolated from 1°, 2°, and 3°-293/PERV supernatants. The primers used for RT-PCR were designed from the PERV-A and PERV-B envelope sequences. Oligonucleotide primers were synthesized homologous to a conserved region at the 3'-end of the *pol* gene (5' of the PERV *env* gene) and a conserved untranslated region 3' of the *env* gene. The amplified products were cloned into the pCRII T-A cloning vector (Invitrogen) and representative clones sequenced from each culture. Unexpectedly, only one PERV envelope gene species was detected in virions produced from all three cultures. The deduced amino acid sequence shows that the surface glycoprotein region of the gene is almost identical to PERV-A, but the transmembrane glycoprotein region of the gene is almost identical to PERV-C (Figure 6). The nucleotide and amino acid sequence homologies of PERV-1.15 with PERV-A, -B, and -C are summarized in Table 6.

While the PERV-1.15 envelope gene is clearly in the PERV-A receptor subgroup family, the gene contains a unique transmembrane glycoprotein that may be important for efficient PERV replication in human cells. The variation in the PERV *env* genes may be significant since variant MLV *env* genes have

been shown to not only broaden the host range of the esotropic MLV but increase the pathogenicity (Rosenberg et al., 1997). These viruses arise by recombination between different endogenous MLVs. Recombination between *env* sequences of exogenous and endogenous feline leukemia viruses (FeLV)

5 also occurs in FeLV-induced thymic tumors.

All 293/PERV cultures contained PERV-A envelope sequences as determined by PCR using subgroup-specific envelope primers. However, PERV-B envelope sequences were not detected in the 293/PERV culture DNAs. The PERV-C envelope sequence was detected in the 1°-293/PERV culture, but not in
10 the subsequent virus passages. PERV-C does not infect most human cells efficiently.

Isolation of Other Human-Tropic PERVs. To identify other human-tropic PERV, primary cell cultures, e.g., pig aortic endothelial cells (PAEC from, for example, NIH and Yucatan minipig breeds), pig PBMC, and primary pig
15 hepatocytes, from normal pig tissues are assayed for the production of human-tropic PERVs on human 293 cells. The cultures are passaged and monitored for PERV production by RT-PCR and RT activity. The PERV populations are passaged onto fresh human cells to generate secondary cultures to determine if a particular PERV species dominates, and if the overall replication efficiency
20 improves. Env subgroups and LTR-U3 sequences are determined as described above.

New preparations of PERV cDNA are synthesized by RT-PCR from virion RNA isolated from PERV sources, and 1°, 2°, and 3°-293/PERV culture supernatants. The region from the virion mRNA polyA tail to the PERV *pol*
25 gene is amplified by RT-PCR. The RT-PCR reactions use a *pol* primer that hybridizes just 5' of the *env* gene, and a second reaction using a *pol* primer homologous to the RT active site region. Two reactions that use different *pol* primers increase the chance of amplifying all PERV variants. ProSTAR Ultra High Fidelity (HF) RT-PCR System (Stratagene) is employed for RT-PCR as it
30 combines high fidelity with long target amplification using the *PfuTurbo* DNA polymerase. *PfuTurbo* DNA polymerase efficiently generates blunt ends. The product is cloned into PCR-Script (Stratagene) designed for blunt-end cloning. This approach does not rely on specific restriction sites to be present in the

PERV sequence, and produces cDNA clones that contain both the envelope gene and the U3 LTR region.

The resulting bacterial colonies are grouped first by envelope subgroups by probing colony lifts on nitrocellulose with ³²P-labeled DNA probes specific for each PERV envelope subgroup. These regions only detect the variable region of the envelope surface glycoprotein. The probing colony lifts with a specific ³²P-labeled probe (DNA region or oligonucleotide), by PCR with specific primers designed to identify the variant sequence, and/or by Southern blots of digested plasmid clone DNA probed with a specific ³²P-labeled probe. In this way, thousands of colonies are easily probed for specific PERV sequences, thereby increasing the detection of rare PERV RNAs. For example, PERV-B was not detected in the DNA in any of the 293/PERV cultures, and PERV-C was barely detected in the DNA of 1°-293/PERV. Thus, PERV-B and PERV-C envelope sequences may be represented in virions, especially in virions produced by PBMC and the 1°-293/PERV culture supernatants. RT-PCR reactions designed to amplify only PERV-B and PERV-C mRNAs are also done. Finally, the expression profile of PERV envelope and U3-LTR sequences in PBMC is analyzed by RNase protection assays. Specific PERV envelope and U3-LTR sequences are cloned into pBluescript KS (Stratagene), and used to produce ³²P-labeled antisense RNA probes. Pig and human GPDH probes are also used to normalize RNA levels in the assays for quantitation of PERV RNA levels in PBMC. These assays determine if PERV-B and PERV-C loci are expressed in PBMC. The nucleotide sequence is then determined for 10-15 representative clones of each unique PERV group.

Cellular DNA is probed, by Southern analysis and PCR, for the presence of any new or variant PERV sequence identified by these experiments. This analysis verifies that the PERV sequence is present in the genome, and not an artifact of the cloning or analysis procedure, although the sequence could be the result of adaptation. The biological properties of all PERV sequences is characterized in an infectious molecular clone. RNase protection assays are used to identify and quantitate the expression levels of the unique PERV elements in RNA isolated from a variety of tissues: brain, pancreas, spleen, liver, heart, kidney, muscle, lung, and PBMC.

Example 3

Molecular Cloning and Characterization of PERVs

A lambda genomic library was prepared from *Sau3A*-partially digested DNA isolated from 2°-293/PERV cells in the Lambda Dash II vector

- 5 (Stratagene). The library was probed with a ³²P-labeled PERV *pol* gene fragment, and ten genomic clones isolated. Six of the clones were unique and contained partial PERV proviruses.

- All of the cloned PERV regions were nearly identical, with at most two amino acid changes in the coding regions, except the PERV sequences in lamA8
10 (Figure 7). The lamA8 *pol* gene contains an 86 nucleotide deletion that results in the truncation of the open reading frame at amino acid 887, a loss of 308 amino acids. The lamA8 *env* gene contains 101 nucleotide insertion that results in the truncation of the open reading frame at amino acid 599, a loss of 60 amino acids which includes the putative transmembrane region of the transmembrane
15 glycoprotein. It is unlikely, therefore, that lamA8 contains a functional *pol* or *env* gene.

- The PERV regions cloned from the 2°-293/PERV culture were compared to the putative complete PERV cDNA, PERV-MSL (Akiyoshi et al., 1998), the PERV-A (Letissier et al., 1997) and PERV-1.15 envelope genes, and gibbon ape
20 leukemia virus (GALV) (Table 7). The 293/PERV *gag* and *pol* genes, as well as the 5' and 3' untranslated regions were nearly identical to the PERV-MSL nucleotide and deduced amino acid sequence.

Table 7. Comparison of the 2°-293/PERV regions contained within the lambda clones with other PERV isolates

	Region	Sequence ^a	Percent Identity			
			PERV-MSL ^b	PERV-A	PERV-1.15	GALV ^c
5	LTR	N	78			
	5'-ut	N	97			
	<i>gag</i>	N	98.9			
		A	98.5			62.6
	<i>pol</i>	N	99			
10		A	99			68
	<i>env</i>	N	86.7	97	99.8	
		A	85	96	99.7	47
	3'-ut	N	100			
15	^a N, nucleotide sequence; A, amino acid sequence.					
	^b PERV-MSL, a putative full-length PERV cDNA clone with the C-type envelope gene.					
	^c GALV, gibbon ape leukemia virus.					
20	ut untranslated region.					

The *env* gene in lamA6 has 4 nucleotide and 2 amino acid differences with PERV-1.15, the 293/PERV *env* gene cloned by RT-PCR from virion RNA (Figure 18). Upon comparing the nucleotide sequence of the 293/PERV and PERV-MSL LTRs, a large divergence was observed in the U3 region (Figure 8). The 293/PERV LTR contains 71 more nucleotides than the PERV-MSL LTR. The sequence of the PERV sequences in Lambda clones A1, A11, A3A, A10, and A8 is also shown in Figure 18.

Two recombinant PERV proviruses were prepared to test if they produce infectious virus in 293 cells. The constructs, an A3A/A6 chimera and an A1/A6 chimera, are outlined in Figure 9. All constructs were built in the pBluescript KS vector (Stratagene), and used the unique BspEI site in the *pol* gene to link the 3'-half of the genome contained in lam A6 to the 5'-half of the genome contained in either lamA1 or lamA3A. A minimum of genomic sequence was cloned with the PERV region: A1, about 1000 bp; A3A, about 30 bp; A6, about 700 bp.

Example 4

Preparation and Detection of Anti-PERV Antibodies

Rabbit polyclonal antisera is produced against 2°-293/PERV virion proteins and against 2°-293/PERV capsid proteins. The 2°-293/PERV cultures produce a relatively large amount of viral particles as demonstrated in Figure 10A. Goat anti-SSAV CA and goat anti-GALV CA antisera easily detect PERV Gag proteins on a Western blot with only minor cross-reaction to control 293 cell supernatant proteins (Figure 10B). These antisera are used as positive controls for the characterization of the anti-PERV antisera and monoclonal antibodies. The 2°-293/PERV cultures produce approximately equal amounts of the p30 capsid (CA) protein in 5 ml of supernatant as ALV infected cultures produce ALV p27 CA protein (lanes 2 and 5). The high-titer ALV stock contained 10^7 iu/ml. The 2°-293/PERV virions are collected and purified by equilibrium density centrifugation on sucrose gradients. The banded virions are collected, dialyzed, and used for antibody production. The PERV CA protein is isolated from SDS-PAGE gels (Figure 10A) and also used for polyclonal antisera production.

Two rabbits are used for each antigen preparation. The antigen (100 µg) mixed in Complete Freund's Adjuvant is used for the initial inoculation. The animal is boosted with 50 µg antigen in Incomplete Freund's Adjuvant on day 14, 21 and 49 after the initial inoculation. The route of injection is normally subcutaneous and/or intramuscular at multiple sites. Test bleeds are drawn on day 35 and 56 and screened against PERV proteins by Western blot and immunoprecipitation assays.

Purified PERV CA protein is used to produce mouse hybridomas that secrete monoclonal antibodies against PERV CA. The PERV capsid protein, purified from polyacrylamide gels, is used as the antigen. Five Balb/c mice are injected intraperitoneally or subcutaneously with 400 µg antigen (per mouse) in Complete Freund's Adjuvant and boosted two weeks later with 250-500 µg of the antigen in Incomplete Freund's Adjuvant. All five mice are then tested for the presence of PERV anti-capsid antibodies by Western blot. One of the positive mice is used for hybridoma preparation. Spleenocytes and/or lymph node cells are removed and fused to non-immunoglobulin secreting myeloma

cell line F/O. Hybridomas are screened and positive hybrids are cloned and then subcloned to obtain monoclonal antibody (MoAb) producing cell lines. The hybridomas are screened by both Western blot and immunoprecipitation of PERV capsid protein. The MoAb cell lines are isotyped and cryopreserved.

- 5 MoAbs are purified from the cell line supernatant by protein A/G column chromatography.

The new anti-PERV antibodies, as well as the anti-SSAV and anti-GALV CA antisera, are tested for their ability to detect PERV proteins in live and fixed PERV infected cells. An immunofluorescence and/or immunohistochemical
10 assay for PERV infection simplifies the detection of PERV infection and allows the direct quantitation of PERV titers.

To detect anti-PERV antibodies in sera, a Western-based assay system is employed. Protein preparations made from PERV virions produced by the 2°-293/PERV culture are separated by SDS-PAGE and transferred to nitrocellulose
15 filters. Virion protein extracts are used for two reasons. First, PERV produced by human cells does not display the α -Gal xenoreactive carbohydrate that may bind to the α -Gal antibodies in human sera. Second, the 2°-293/PERV culture produces high levels of PERV virions as shown by analysis of PERV virion protein extracts. Goat anti-SSAV and anti-GALV CA antisera that cross-reacts
20 with the PERV CA protein is employed. The 2°-293/PERV virions are purified by equilibrium centrifugation on sucrose gradients. In one embodiment, the assay employs peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Amersham, Pharmacia Biotech, Piscataway, NJ) to detect antibodies bound to the PERV proteins. To determine what effect human sera
25 may have on the performance of the assay (e.g., alter the antibody sensitivity or cross-reaction with common human antibodies), the anti-CA antisera is added to dilutions of control human sera (1:10, 1:50, 1:100) and tested.

Serum samples from at least 300 pig slaughterhouse workers, plus controls, are screened. Control samples, including sera from a patient with little
30 or no exposure to pigs, and sera from two populations of retrovirus-infected patient populations (50 HIV-1; 20 HTLV-1) is used to evaluate the specificity of antibody binding to PERV and non-PERV proteins. Preparative single well SDS-PAGE is used to separate the PERV virion proteins, and the proteins

transferred to a nitrocellulose filter. The Miniblotter II apparatus (Immunitics, Cambridge, MA) permits the screening of 45 different samples by dividing the Western blot into 45 sample cells. A negative control serum and a positive control serum (human sera with SSAV or GALV anti-capsid antisera) is assayed with each blot. A 1:50 dilution of the serum samples is screened initially. Human blood samples are also tested for PERV nucleic acid and anti-PERV antibodies.

Example 5

10 Soluble Forms of the Subgroup A Avian Leukosis Virus Receptor Tva Significantly Inhibit ALV(A) Infection *in vitro* and *in vivo*

Three cell surface proteins have been identified as ALV receptors: Tva, the receptor for ALV(A) (Bates et al., 1998; Bates et al., 1993; Young et al., 1993); CAR1, the receptor for ALV(B) and ALV(D) (Brojatsch et al., 1996; 15 Smith et al., 1998); and SEAR, the receptor for ALV(E) (Adkins et al., 1997). To aid in the characterization of the interactions between Tva and ALV(A) envelope glycoproteins, soluble forms of the 83 amino acid extracellular domain of the Tva receptor protein (sTva) were constructed by Young and colleagues (Connolly et al., 1995), who reported that pre-incubation of the sTva proteins 20 with different envelope subgroup ALVs caused a specific block to infection of susceptible chicken cells by ALV(A), but had no effect on ALV(B) or ALV(C) infection.

To determine if cells and chickens expressing sTva proteins are resistant to ALV(A) infection, ALV-based replication-competent retroviral vectors were 25 used to efficiently deliver and express *sTva* genes (Federspiel et al., 1997). The vectors are available with five different envelope subgroups (A-E) which enables multiple genes to be delivered and expressed in virtually every cell.

Materials and Methods

Soluble receptor and retroviral vector constructs. The two soluble 30 receptor gene constructs, contained in the plasmids pLC126 and pKZ457, were gifts of John Young (Harvard Medical School). The pLC126 *sTva* gene (Connolly et al., 1994; Hughes et al., 1987), encoding the 83-amino-acid Tva extracellular domain fused to a 9-amino-acid antibody epitope tag derived from

influenza virus hemagglutinin, followed by six histidine residues, was isolated as a *NcoI-PstI* fragment and cloned into the *NcoI* and *PstI* sites of the CLA12NCO adaptor plasmid (Federspiel et al., 1997; Hughes et al., 1987). The pKZ457 *stva-mIgG* gene, encoding the 83-amino-acid Tva extracellular domain fused to the constant region of the mouse IgG heavy chain (nucleotides 353-1072) (Tucker et al., 1979), was isolated as a *NcoI-BlpI* fragment. The *BlpI* site was made blunt and the modified fragment was cloned into the *NcoI* and *SmaI* sites of CLA12NCO. Both the *stva* and *stva-mIgG* genes had been modified to contain *NcoI* sites at their initiator ATGs. The soluble receptor gene cassettes were isolated as *ClaI* fragments from the adaptor plasmids and cloned into the unique *ClaI* site of the RCASBP, RCAS, and RCOSBP retroviral vectors with subgroup (B) and subgroup (C) envelope genes. The RCAS family of replication-competent retroviral vectors have been described (Federspiel et al., 1994; Federspiel et al., 1997; Hughes et al., 1987; Petropoulos et al., 1991; Petropoulos et al., 1992).

The *stva-mIgG* gene isolated as a *ClaI* fragment from the CLA12NCO adaptor plasmid was subcloned into the TFANEO expression vector (Federspiel et al., 1989). TFANEO is a companion expression vector to the RCAS family of retroviral vectors. The expression cassette of TFANEO consists of two LTRs derived from the RCAS vector that provide strong promoter, enhancer, and polyadenylation sites flanking a unique *ClaI* insertion site. The TFANEO plasmid also contains a *neo* resistance gene expressed under the control of the chicken β -actin promoter, and an ampicillin resistance gene for selection in *E. coli*.

The RCASBP(A)AP, RCASBP(C)AP and RCASBP(C)AP retroviral vectors which contain the heat-stable human placental alkaline phosphatase gene (AP) have been described (Federspiel et al., 1997; Field-Berry et al., 1992; Fekete et al., 1993). The AP gene, contained on a *SalI* fragment, was cloned into the *SalI* site of the CLA12 adaptor plasmid, and then subcloned into the RCASBP vectors as a *ClaI* fragment (gift of Constance Cepko).

Cell culture and virus propagation. Chicken embryo fibroblasts (CEFs) derived from 10-day, line 0 embryos (C/E) (Astrin et al., 1979) were grown in DMEM (GIBCO/BRL) supplemented with 10% tryptose phosphate broth

(GIBCO/BRL), 5% fetal bovine serum (GIBCO/BRL), 5% newborn calf serum (GIBCO/BRL), 100 units of penicillin per ml, and 100 µg of streptomycin per ml (Quality Biological, Inc., Gaithersburg, MD) as previously described (Federspiel et al., 1997). DF-1 cells were grown in DMEM supplemented with
5 10% fetal bovine serum, 100 units of penicillin per ml, and 100 µg of streptomycin per ml (Himly et al., 1998; Schaefer-Klein et al., 1998). Both CEF and DF-1 cultures were passages 1:3 when confluent.

Virus propagation was initiated by calcium phosphate transfection of plasmid DNA that contained the retroviral vector in proviral form (Federspiel et al., 1997). In standard transfections, 5 µg of purified plasmid DNA was
10 introduced into DF-1 cells or early passage CEF by the calcium phosphate precipitation method (Kingston et al., 1989). Viral spread was monitored by assaying culture supernatants for ALV capsid protein by either Western transfer analysis or ELISA (Smith et al., 1979). Virus stocks were generated from the
15 cell supernatants. The supernatants were cleared of cellular debris by centrifugation at $2000 \times g$ for 10 minutes at 4°C and stored in aliquots at -80°C. DF-1 cells transfected with the TFANEO plasmid were grown in 500 µg/ml G418 (Gibco/BRL) to select for neomycin-resistant cells. Clones were isolated using cloning cylinders (Bellco Glass Inc., Vineland, NJ), expanded, and
20 maintained with standard medium supplemented with 250 µg/ml G418.

ALV alkaline phosphatase challenge assay. In a direct AP challenge assay, CEF or DF-1 cell cultures (about 30% confluent) were incubated with 10-fold serial dilutions of the RCASBP/AP virus stocks for 36-48 hours at 39°C. In a pre-absorption AP challenge assay, the 10-fold viral serial dilutions were first
25 mixed with 2 ml of supernatant containing sTva-mIgG for 3 hours at 4°C, and then assayed as above. The assay for alkaline phosphatase activity was modified from procedures of Cepko and co-workers (Federspiel et al., 1994; Fekete et al., 1993; Fields-Berry et al., 1995). Cells were fixed in 4% paraformaldehyde in Dulbecco's phosphate-buffered saline (PBS) for 30 minutes at 25°C, washed
30 twice in PBS for 5 minutes each, and incubated for 1 hour at 65°C to inactivate endogenous AP activity. The cells were then washed twice with AP detection buffer (100 mM Tris•Cl, pH 9.5/100 mM NaCl/50 mM MgCl₂) for 10 minutes and exposed to the AP chromogenic substrates nitroblue tetrazolium (300 µg/ml)

and 5-bromo-4-chloro-3-indoyl phosphate (170 µg/ml) (GIBCO/BRL).

Enzymatically active AP produces an insoluble purple precipitate. The reaction was stopped by the addition of 20 mM EDTA, pH 8.0 in PBS.

Immunoprecipitation and Western transfer analysis of stva-mIgG

- 5 proteins. A 500 µl aliquot of culture supernatant or serum was incubated with 50 µl of anti-mouse IgG-agarose beads (Sigma) for ≥ 1 hour at 4°C. The sTva-mIgG agarose bead complexes were collected by centrifugation and washed twice in dilution buffer [50 mM Tris-buffered saline (TBS), 1% Triton X-100, 1 mg/ml BSA], once in 50 mM TBS and once in 0.05 M Tris•Cl, pH 6.8. The
- 10 washed complexes were collected by centrifugation, resuspended in 50 µl 1X Laemmli buffer (2% SDS, 10% glycerol, 0.05 M Tris•Cl, pH 6.8, 0.1% bromophenol blue) without β-mercaptoethanol, and heated for 5 minutes at 100°C. The agarose in the samples was collected by centrifugation for 2 minutes and the supernatants were transferred to new tubes. Prior to gel electrophoresis,
- 15 1.0 µl β-mercaptoethanol was added to each 50 µl sample and the samples were heated for 5 minutes at 100°C. The denatured immunoprecipitates were separated by 12% SDS-PAGE, and transferred to a nitrocellulose membrane. The filters were blocked with 10% non-fat dry milk (NFDM) in PBS, probed with 0.05 µg/ml peroxidase-conjugated goat anti-mouse IgG antibodies
- 20 (Kirkegaard and Perry laboratories, Gaithersburg, MD) in rinse buffer (100 mM NaCl, 10 mM Tris•Cl, pH 8, 1 mM EDTA, 0.1% Tween 20) and 1% NFDM, and washed in rinse buffer. Protein/antibody complexes were detected with the Western Blot Chemiluminescence Reagent (NEN) according to the manufacturer's instructions. The immunoblot was then exposed to Kodak X-
- 25 Omat film.

- In vivo ALV challenge assay. Line 0 embryos were somatically infected with RCASBP(B), RCASBP(B)stva, or RCASBP(B)stva-mIgG by injecting unincubated eggs near the blastoderm with 100 µl containing 1×10^6 CEF or DF-1 cells producing the virus. Line 0 is a White Leghorn line that is
- 30 genetically susceptible to all ALV subgroups except subgroup (E) and is free of endogenous proviruses that are closely related with ALV (Astrin et al., 1979). Viremic chicks were identified at hatch by ELISA for the ALV capsid protein p27. Viremic and uninfected control chicks were infected intra-abdominally

with 10^5 infectious units of either RAV-1 [an ALV(A) isolate] or RAV-49 [an ALV(C) isolate]. Blood was collected at 2, 4 or 9 weeks post-challenge, and the serum assayed for infectious subgroup (A), (B), or (C) ALV by the *in vitro* ALV Assay (see below).

- 5 *In vitro* ALV assay. The presence of infectious ALV in chickens was determined by assaying the serum samples on a panel of cell lines with different ALV envelope subgroup susceptibilities. The panel of indicator cell lines included: line 0 CEF (C/E) which supports the replication of ALV(A), ALV(B), and ALV(C); line *alv6* CEF (C/A) which supports ALV(B) and ALV(C)
- 10 replication; line RP30B-cell line (C/B) which supports ALV(A) and ALV(C) replication; and line 15.C-12 CEF (C/C) which supports ALV(A) and ALV(B) replication. Serum samples (100 μ l) were added to the cells and the cells were incubated for 9 days in media (5% serum) to allow ALV to spread. The media was changed after 3 days to avoid detection of ALV proteins in the original
- 15 serum sample. The cells were then solubilized by 2 cycles of rapid freeze-thaw to release ALV Gag antigens. The ALV capsid protein was detected by ELISA. A positive sample was defined as having an optical density reading of > 0.200 . The *in vitro* ALV Assay can detect infectious ALV titers as low as 10 IFU/ml.

- RNase protection assay. Total RNA was isolated from cells in culture, or
- 20 homogenized tissues of experimental birds, by the RNazol B method (Tel-Test, Inc., Friendswood, TX). Sequence-specific RNA probes were cloned into pBluescript KS as follows: the RAV-1 envelope sequences were cloned as an *Xho*I to *Xba*I fragment (Genbank accession # M19113; nucleotides 248-676) (Bora et al., 1988); the RAV-2 envelope sequences were cloned as a *Bam*HI to
- 25 *Sal*I fragment (Genbank accession # M14902; nucleotides 612-1080) (Bora et al., 1986); the *stva* probe was generated from an *Eco*RI to *Pst*I fragment from the plasmid pLC126 (Genbank accession # L22752; nucleotides 9-386) (Bates et al., 1993; Connolly et al., 1994); and the *stva-mIgG* probe was generated from a
- 30 *Clal* to *Bam*HI fragment derived from the adaptor plasmid construct which contains the 5'-*Clal* site and transcription leader from the CLA12NCO adaptor plasmid and a synthetic sequence encoding the 83-amino acid Tva extracellular domain from pKZ457 that is different from the *stva* gene. A fragment of the chicken glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (Genbank

accession # K01458; nucleotides 163-361) (Panabieres et al., 1984) was used as a control for the quantity and quality of the RNA. The constructs were linearized by restriction endonuclease digestion and gel purified. ³²P-labeled antisense RNA probes were synthesized using the RNA Transcription Kit (Stratagene, La Jolla, CA). The probes were hybridized with 20 µg of total RNA in 20 µl hybridization solution (80% formamide, 10 mM sodium citrate pH 6.4, 300 mM sodium acetate pH 6.4, 1 mM EDTA) overnight at 42°C. RNase protection assays were performed using the RPA II Ribonuclease Protection Kit (Ambion, Austin, TX). The RNA samples were digested with the RNase A/T1 mixture diluted 1:75. The protected RNA probe fragments were separated on a 6% acrylamide/7.6 M urea gel and exposed to Kodak X-Omat film.

PCR assays. DNA was isolated from cells in culture or tissues of experimental birds using the QIAmp Tissue Kit (Qiagen). Each PCR contained 1.25 µl 10X PCR buffer (final concentration, 50 mM Tris•Cl, pH 8.3, 50 mM KCl, 7 mM MgCl₂, 1.1 mM β-mercaptoethanol), 1.25 µl of 1.7 mg/ml BSA, 0.5 µl of each dNTP at 25 mM, 0.5 µl of each primer ($A_{260} = 5$), 6.0 µl H₂O, and 1.0 µl of DNA (genomic DNA about 100 ng/µl; plasmid DNA about 2 ng/µl). The reactions were heated to 90°C for 1 minute and initiated by the addition of 1.5 µl of Taq DNA polymerase (Promega, Madison, WI) diluted 1:10 v/v (0.75 units). Thirty cycles of PCR were carried out as follows: 90°C for 40 seconds, then 59°C for 80 seconds. Diagnostic primers used to detect ALV(A) *env* (Bora et al., 1988) were 5'-GGGACGAGGTTATGCCGCTG-3' (SEQ ID NO:24; about 50 bp upstream of *Kpn*I site) and 5'-GGGCGTGCGCGCATTACCAC-3' (SEQ ID NO:25; nucleotides 871-851), yielding a 937 bp fragment. The PCR extension temperature was increased to 62°C for amplifying ALV(A) *env*. Diagnostic primers used to detect ALV(B) *env* (Bora et al., 1986) were 5'-GACCGACCCAGGAACAATC-3' (SEQ ID NO:26; nucleotides 713-732) and 5-ATGAGGAAAATTGCGGGTGG-3' (SEQ ID NO:27; nucleotides 1141-1122), yielding a 429 bp fragment. Diagnostic primers used to detect *stva* (Bates et al., 1993; Connolly et al., 1994) were 5'-GGAATGTGACTGGTAATGGA-3' (SEQ ID NO:28; nucleotides 56-75) and 5'-GCCTTAGTGATGGTGATGGT-3' (SEQ ID NO:29; nucleotides 369-350), yielding a 314 bp fragment. Diagnostic primers used to detect *stva-mIgG* were 5'-CCATCCGTCTTCATCTTCCCT-3'

(SEQ ID NO:30; nucleotides 974-994) and 5'-
 TGGTGC GGTGTCCTTGTAGTT-3' (SEQ ID NO:31; nucleotides 1562-1542),
 yielding a 589 bp fragment of the mouse IgG gene (Tucker et al., 1979). The
 amplified DNA fragments were separated on 0.8% agarose gels and visualized
 5 with ethidium bromide.

Results

Experimental approach. The *stva* and *stva-mIgG* receptor gene fusions
 were subcloned into the CLA12NCO adaptor plasmid which contains a
 transcriptional leader sequence and has a consensus ATG start site contained in a
 10 *NcoI* site. These sequences work very efficiently with the promoter/enhancer
 elements of the ALV-based retroviral vectors to express experimental genes at
 high levels (Hughes et al., 1987). The RCAS family of retroviral vectors were
 derived from the Schmidt-Ruppin A strain of Rous sarcoma virus (RSV) and
 were present in proviral form on pBR-based plasmids (Federspiel et al., 1997).
 15 Experimental genes were inserted into the vectors in the unique *ClaI* site (which
 replaces the *src* gene in RSV) and so are translated from a spliced mRNA.
 Retroviral vectors that carry and express the *stva* and *stva-mIgG* genes are shown
 schematically in Figure 11. Virus propagation was initiated by transfection of
 plasmid DNA containing the retroviral vector into avian cells (Figure 12). The
 20 culture was then passaged until a maximum viral titer was achieved (6-10 cell
 passages depending on the vector (Federspiel et al., 1997). Because vectors that
 use different receptors are available, this system can be used to deliver multiple
 genes to virtually all cells in the culture (Givol et al., 1994). Cell cultures that
 express sTva or sTva-mIgG from a subgroup (B) or (C) vector were
 25 subsequently challenged with ALV(A) to quantitate the antiviral effect of the
 sTva proteins.

Antiviral effect of sTva *in vitro* and *in vivo*. The initial experiments
 testing the effects of soluble receptors on viral replication were done with the
 sTva protein. The *stva* gene was introduced into the RCASBP vector, which
 30 produces the highest titer viral stocks and the highest level of expression of an
 experimental. To quantitate the antiviral effect of sTva on ALV(A) infection,
 CEF fully infected with the vector alone [either RCASBP(B) or RCASBP(C)],
 vectors that express the *stva* gene [RCASBP(B)*stva* or RCASBP(C)*stva*] or

uninfected CEF were challenged with RCASBP(A)AP, the subgroup (A) RCASBP vector containing the human placental alkaline phosphatase reporter gene (AP). The results of a representative assay are shown in Table 8. CEF cultures producing sTva, either from RCASBP(B) or RCASBP(C), were > 100-
 5 fold more resistant to infection by RCASBP(A)AP than cultures infected with the vector alone. CEF cultures infected by the RCASBP(B) or RCASBP(C) vector alone were 3-5-fold less susceptible to RCASBP(A)AP. The antiviral effect of sTva was specific for RCASBP(A)AP infection, since infection by viruses with other envelope subgroups were not inhibited (Table 8).

10

Table 8.
 Relative resistance of CEF expressing sTVA or sTVA-mIgG to ALV infection.

		Challenge Virus		
		RCASBP(A)AP ^a	RCASBP(B)AP	RCASBP(C)AP
15	Uninfected CEF	1.5×10^6	2.1×10^5	8.4×10^4
	RCASBP(B)	4.3×10^5 (4) ^b	ND ^c	2.0×10^4 (4.2)
	RCASBP(B)stva	2.9×10^3 (517)	ND	2.1×10^4 (4.0)
	RCASBP(C)	6.0×10^5 (3)	8.3×10^4 (2.5)	ND
	RCASBP(C)stva	4.0×10^3 (375)	1.2×10^5 (1.8)	ND
20	RCASBP(C)stva-mIgG	1.5×10^3 (1000)	8.0×10^4 (2.6)	ND

^aAP-human placental alkaline phosphatase reporter gene.

^bThe resistance of experimental and control CEF to ALV infection relative to uninfected CEF is given in parentheses.

^cND-not done

25

The CEF cultures infected with RCASBP(B) and RCASBP(B)stva were used to inoculate unincubated line 0 eggs to produce chicks viremic with RCASBP(B) or RCASBP(B)stva. Viremic chicks produced in this manner are tolerant to most ALV antigens since the early embryo was infected. The chicks
 30 were challenged with 10^5 infectious units of RAV-1, an aggressive ALV(A) strain, to quantitate the antiviral effect of sTva. Blood samples were collected from representative birds of each group at 2 weeks post-challenge, and from all birds 9 weeks post-challenge. The sera were assayed for ALV(A) and ALV(B) by the *in vitro* ALV Assay. The results of the challenges are summarized in

Table 9. Ninety-six percent of the birds infected with the RCASBP(B) vector alone and then challenged with RAV-1 produced ALV(A) at both experimental time points as expected. However, 95% of the birds infected with RCASBP(B)stva did not produce detectable levels of ALV(A). These results demonstrate that sTva has a strong antiviral effect on ALV(A) infection both *in vitro* and *in vivo*. These results also demonstrate the utility of using vectors with different subgroups *in vivo* since experimental birds could be infected with both the RCASBP(B) vector and RAV-1. However, the level of sTva expression could not be quantitated since neither the hemagglutinin nor the histidine epitope tags included on the sTva protein allowed efficient immunoprecipitation of the protein.

Table 9. Chickens expressing sTva are resistant to ALV(A) infection.

	Virus Subgroup Detected					
	2 Weeks ^a			9 Weeks		
	A&B	B	A	A&B	B	A
Uninfected	0/10	0/10	0/10	0/9	0/9	0/9
RCASBP(B)	9/9	9/9	0/9	6/6	6/6	0/6
RCASBP(B)+RAV-1	10/10	10/10	9/10	15/15	15/15	15/15
RCASBP(B)stva	10/10	10/10	1/10	11/12	11/12	0/12
RCASBP(B)stva+RAV-1	10/10	10/10	0/10	20/20	20/20	1/20

^aAll of the birds in each group were not necessarily assayed at both time points.

Antiviral effect of sTva-mIgG *in vitro*. Although the tagged version of sTva could not be immunoprecipitated efficiently, an sTva immunoadhesin sTva-mIgG can be immunoprecipitated and quantitated. sTva-mIgG consists of the 83-amino acid Tva extracellular domain fused to the constant region of the mouse IgG heavy chain. The *stva-mIgG* gene was introduced into the RCASBP(C) vector. To quantitate the antiviral effect of sTva-mIgG compared to sTva, CEF cultures infected with RCASBP(C)stva-mIgG, RCASBP(C)stva, or RCASBP(C) were challenged with either RCASBP(A)AP or RCASBP(B)AP. CEF expressing sTva-mIgG were about 300-fold more resistant to

RCASBP(A)AP infection compared to cells infected with the vector alone, and 2- to 3-fold more resistant than cells expressing sTva (Table 8). The antiviral effect was specific for ALV(A) since no significant change in susceptibility was observed when the cultures were challenged with RCASBP(B)AP.

- 5 ALV replication in a permanent, non-transformed cell line derived from line 0 CEF called DF-1 has been described (Himly et al., 1998; Schaefer-Klein et al., 1998). ALV and ALV-based retroviral vectors replicate and express inserted genes in DF-1 cells at levels similar to CEF, and DF-1 can be used to generate clonal cell lines. The antiviral effect of sTva-mIgG produced in DF-1 cultures
- 10 infected with RCASBP(C)stva-mIgG (Table 10) was similar to that seen in CEF cultures (Table 8). The sTva-mIgG protein was immunoprecipitated from cell culture supernatants with anti-mouse IgG antibody conjugated to agarose beads and analyzed by Western transfer of SDS-PAGE gels (Figure 13). The immunoprecipitated sTva-mIgG protein migrates as a broad band (50-60 kDa)
- 15 due to post-translational modification and as a minor about 38 kDa band. The about 38 kDa band is probably a degradation product of sTva-mIgG since both bands appear after immunoprecipitation with an ALV(A) surface glycoprotein immunoadhesin, and the amount of the about 38 kDa band increases after repeated freeze-thaw cycles of the viral supernatants. Stable clonal DF-1 cell
- 20 lines were generated that express different levels of sTva-mIgG under the control of the TFANEO expression vector. These cell lines do not produce infectious ALV and are resistant to RCASBP(A)AP infection at levels similar to cultures expressing sTva-mIgG from the retroviral vectors. Therefore, chronic ALV infection does not make a major contribution to the antiviral effect obtained.

- 25 Relationship between sTva-mIgG expression level and the antiviral effect. The *stva-mIgG* gene was subcloned into the RCAS(C) and RCOSBP(C) retroviral vectors. Previously, it has been reported that the RCAS vector replicates to a 5-15-fold lower titer (depending on envelope subgroup and inserted gene) compared to RCASBP and produces protein at an equivalently
- 30 reduced level (Federspiel et al., 1994). The RCOSBP vector, which lacks a strong transcription enhancer in the LTR, replicates to about 100-fold lower titer compared to RCASBP and produces lower levels of protein. DF-1 cultures infected with RCASBP(C)stva-mIgG, RCAS(C)stva-mIgG, or RCOSBP(C)stva-

mIgG were challenged with RCSABP(A)AP to determine the antiviral effect of different levels of sTva-mIgG on ALV(A) infection. The results of a representative assay are shown in Table 10. The sTva-mIgG produced by these DF-1 cultures was detected by immunoprecipitation of sTva-mIgG followed by the analysis of the proteins by Western transfer (Figure 13). As expected, cultures infected with RCASBP produced the highest level of sTva-mIgG and the greatest antiviral effect (about 200-fold). Cultures infected with RCAS produced slightly lower levels of sTva-mIgG protein and a lower antiviral effect (about 100-fold). Finally, cultures infected with RCOSBP produced the lowest level of sTva-mIgG protein and a modest antiviral effect (about 15-fold).

Table 10.

Relative resistance of DF-1 cells expressing sTva-mIgG to ALV(A) infection.

	RCASBP(A)AP	Resistance ^a
Uninfected DF-1	3.3×10^6	---
15 RCASBP(C)	1.3×10^6	3
RCASBP(C)stva-mIgG	5.3×10^3	622
RCAS(C)	6.4×10^5	5
RCAS(C)stva-mIgG	5.7×10^3	579
RCOSBP(C)	9.0×10^5	4
20 RCOSBP(C)stva-mIgG	5.2×10^4	63

^aThe resistance of the cells to ALV(A) infection was determined by dividing the titer obtained on the control uninfected DF-1 cells by the titer obtained for each experimental group.

The antiviral effect of sTva and sTva-mIgG on ALV(A) infection may represent the minimum antiviral effect attainable *in vitro* as measured by the direct ALV AP challenge assay. The assays were done on subconfluent cell cultures (30%) where the levels of the soluble receptor protein had not accumulated to the levels expressed by a confluent culture. To determine the antiviral effect of higher levels of sTva-mIgG, RCASBP(A)AP was pretreated with supernatants collected from confluent DF-1 cultures infected with RCASBP(B), RCASBP(B)stva-mIgG, RCOSBP(B), or RCOSBP(B)stva-mIgG, and then assayed as before. Preabsorption of RCASBP(A)AP with high levels of

sTva-mIgG significantly increased the antiviral effect compared to a direct assay: RCASBP(B)stva-mIgG pretreatment increased the antiviral effect of the direct assay about 500-fold; and RCOSBP(B)stva-mIgG pretreatment increased the direct antiviral effect about 60-fold.

- 5 Delivery and expression of sTva and sTva-mIgG *in vivo*. To characterize the efficiency of RCASBP delivery and expression of the sTva proteins in chickens, unincubated line 0 eggs were injected with CEF producing the RCASBP(B) or RCASBP(C) vectors alone, the vectors with the *stva* gene, or the vectors with the *stva-mIgG* gene. Viremic chicks were identified on the day of
- 10 hatch by an ELISA assay for ALV capsid protein. The sTva-mIgG protein was immunoprecipitated from serum samples of both RCASBP(B)stva-mIgG and RCASBP(C)stva-mIgG infected birds and visualized by Western analysis (Figure 14). The *stva*, *stva-mIgG*, and RCASBP(B) *env* RNA expression levels in liver, heart, spleen, bursa, thymus, kidney, and muscle tissues of infected birds
- 15 were analyzed by RNase protection assay. An RNase protection analysis of a representative bird infected with RCASBP(B)stva and a representative bird infected with RCASBP(B)stva-mIgG are shown in Figure 15. Relatively high levels of the *stva* or *stva-mIgG* and ALV(B) *env* RNAs were detected in all tissues assayed, indicating that the inserted genes were delivered and expressed
- 20 efficiently by the RCASBP(B) vector.

Table 11.

Chickens expressing either sTva or sTva-mIgG are resistant to ALV(A) infection but not ALV(C) infection.

	Virus Subgroup Detected ^a		
	B	A	C
5			
	RCASBP(B)+RAV-1	4/4	4/4
	RCASBP(B)stva+RAV-1	5/5	0/5
	RCASBP(B)stva-mIgG+RAV-1	9/9	0/9
10	RCASBP(B)+RAV-49	4/4	3/4
	RCASBP(B)stva+RAV-49	4/4	4/4
	RCASBP(B)stva-mIgG+RAV-49	7/8	6/8

^a Assays were done 4 weeks after challenge.

- 15 Antiviral effect of sTva-mIgG *in vivo*. Chicks infected with the RCASBP(B) vector alone, RCASBP(B)stva, or RCASBP(B)stva-mIgG were split into two groups and challenged with 10⁵ infectious units of either RAV-1 (subgroup A) or RAV-49 (subgroup C). Blood was collected from each bird four weeks after challenge, and the serum was assayed for ALV(A), ALV(B) and
- 20 ALV(C) by the *in vitro* ALV Assay (Table 11). As expected, ALV(B) was detected in virtually all of the birds since the RCASBP(B) vector was used for gene delivery. ALV(A) was not detected in the serum of RAV-1 challenged birds containing the *stva* or the *stva-mIgG* genes. However, ALV(A) was detected in the serum of the birds infected with the RCASBP(B) vector alone
- 25 and challenged with RAV-1. In contrast, the birds of all three experimental groups were equally susceptible to RAV-49 challenge as shown by the presence of ALV(C) in the majority of the birds. Since 19% of the birds challenged with RAV-49 did not produce detectable levels of ALV(C), the titer of the RAV-49 stock may have been lower than expected. The antiviral effect of sTva and sTva-
- 30 mIgG was specific for ALV(A), consistent with the proposed mechanism of antiviral action, receptor interference.

Representative birds from each RAV-1 challenged experimental group were analyzed for the presence of ALV(A) and ALV(B) *env*, *stva* and *stva-mIgG*

sequences in RNA and DNA isolated from a variety of tissue samples of each bird. A low level of RAV-1 infection and replication in a subset of tissues may go undetected by the *in vitro* ALV assay due to virus inactivation by the sTva or sTva-mIgG proteins in the serum. Antisense riboprobes and primer sets were developed to specifically detect each target sequence by RNase protection assay and PCR. RNA and DNA were isolated from liver, heart, spleen, bursa, thymus, kidney, and muscle tissue of each bird and analyzed by RNase protection assay and PCR assay. A representative RNase protection assay of RNA of one tissue (bursa) from a bird of each experimental group, and an uninfected control bird, is shown in Figure 16. A representative PCR analysis of DNA isolated from tissues of a RAV-1 challenged bird from each experimental group is shown in Figure 17. RAV-1 RNA and DNA were only detected in tissues of birds infected with the RCASBP(B) vector challenged with RAV-1. Therefore, the expression of sTva and sTva-mIgG significantly reduces, if not eliminates, infection by the ALV(A) strain RAV-1 in chickens.

Discussion

Cells expressing the sTva proteins showed significant resistance to ALV(A) infection, presumably due to the secreted receptor proteins binding the glycoproteins of the invading virion, blocking the interactions of the virus and the membrane bound Tva, a form of receptor interference. Tva has been hypothesized to be necessary and sufficient to mediate ALV(A) entry (Balliet et al., 1998; Bates et al., 1993). Several possible mechanisms could account for the sTva inhibition of ALV(A) entry sTva binding of an ALV(A) surface glycoprotein may lead to an irreversible conformational change in SU and TM. Several studies have shown that sTva binding to purified ALV(A) envelope glycoproteins induces a temperature-dependent conformational change in the glycoproteins, and appears to convert the envelope glycoproteins to a membrane-binding state (Balliet et al., 1999; Damico et al., 1999; Gilbert et al., 1995; Hernandez et al., 1994). Binding of sTva or sTva-mIgG to the envelope glycoproteins on the surface of the virus induces a conformational change in both SU and TM similar to the events leading to fusion of the viral and host cell membranes, and converts SU and TM into a form that is unable to bind Tva on the surface of the cell. A conformational change may also lead to the loss of

some of the SU subunits (Orloff et al., 1993). Finally, sTva may inhibit ALV(A) entry by simply binding to SU and physically blocking the access of membrane bound Tva to the virion. By whatever mechanism(s), the sTva proteins block the entry of ALV(A) into cultured cells and cells and tissues of chickens.

- 5 The replication-competent ALV-based retroviral vector experimental system enabled the efficient delivery and expression of the *stva* and *stva-mIgG* genes both in cultured cells and in virtually all the cells and tissues of the chicken. RCASBP(A) and (RCASBP(B), as well as other combinations of ALV retroviral vectors [ALV(B) followed by ALV(A); ALV(C) followed by
- 10 AVL(A)], can be used in CEF and DF-1 cells *in vitro* and *in vivo*. As reported previously, the replication of some RCASBP(B) viruses in CEF and DF-1 cells, and RCASBP(C) viruses on DF-1 cells, were somewhat cytopathic (Himly et al., 1998; Schaefer-Klein et al., 1998). The cytopathic effect manifests itself as a
- 15 pause in growth rate (2-6 days), after which the cells recover, divide at a normal rate, and express the viral and experimental proteins. It appears that only the cells that produce high levels of the RCASBP(B) or RCASBP(C) envelope glycoproteins show the cytopathic effects. The replication of subgroup (B) and (C) RCAS and RCOSBP viruses, which replicate to lower titers compared to RCASBP, do not cause detectable cytopathic effects. Chronic infection of CEF
- 20 and DF-1 cells with an ALV vector results in a low level of resistance to infection by other ALV *env* subgroup vectors (2-5-fold) compared to uninfected cells. This may indicate that while the ALV glycoproteins specifically and efficiently interact with the appropriate receptor resulting in receptor
- 25 interference, the high level expression of one type of envelope glycoprotein on the cell surface may interfere either directly or indirectly with the ability of other ALVs to interact with their host receptors.

- Both the RCASBP(B) and RCASBP(C) retroviral vectors were efficient in generating viremic chicks without detectable pathologic effects in short term infections, and the infected chicks expressed relatively high levels of sTva-mIgG
- 30 protein in their serum. Chicks infected with RCASBP(B) were also efficiently infected with ALV(A). The RCASBP(B) vector efficiently delivered and expressed the *stv-a* and *stva-mIgG* genes in all tissues tested, and resulted in a significant antiviral effect on ALV(A) infection and replication. By delivering

the *stva* genes in the early embryo, the immune system of the chicken can be evaded. The birds are tolerized to the sTva and sTva-mIgG proteins, and to most of the ALV antigens since the viral vector used to deliver *stva* and *stva-mIgG* and the challenge viruses are virtually identical except for small regions of SU.

- 5 The expression of the sTva and sTva-mIgG proteins *in vivo* allowed the examination of the effects of the viral glycoprotein-soluble receptor interactions in a wide variety of cells. One RAV-1 challenged RCASBP(B)stva infected bird did contain infectious ALV(A) in its serum. Unfortunately the bird died of non-viral causes before tissues could be obtained.
- 10 CD4, an important cell-surface protein of the T-lymphocytes, is the primary receptor for HIV-1. Several groups developed and expressed soluble forms of CD4 (sCD4) and demonstrated that recombinant sCD4 proteins could bind specifically to HIV-1 envelope glycoproteins and inhibit HIV-1 infection *in vitro* (Daar et al., 1990; Harbison et al., 1990; Klasse et al., 1993; Orloff et al., 1993; Schacker et al., 1995; Weiss, 1992). For injection of a recombinant
- 15 antiviral protein to be effective against cell to cell transmission of the virus, it may be necessary to use a gene therapy approach in which target cells actively express the soluble receptor. The gene therapy approach has been tested for HIV-1: a sCD4 gene construct was expressed by a murine leukemia virus-based retroviral vector in human T-cell lines and in primary peripheral blood
- 20 lymphocytes (Morgan et al., 1994). In cell culture populations engineered to express sCD4 (30-50% of the cells contained the sCD4 gene) HIV-1 replication was inhibited 50-70% indicating that a sCD4 antiviral approach against HIV-1 might be more effective. Since the initial sCD4 studies were published, the
- 25 chemokine receptors have been identified as co-receptors necessary for efficient HIV-1 entry into cells (Hunter, 1997). Since both CD4 and a chemokine receptor are required for efficient HIV-1 entry into cells, sCD4 alone may not be an effective inhibitor of HIV-1 entry.

- The results clearly indicate that a soluble receptor interference antiviral
- 30 strategy can effectively block the replication of at least some retroviruses, and that this approach may be applicable to other virus groups that require specific viral glycoprotein-host receptor interactions for entry into the cell. The application of this strategy to protect animals against specific viral diseases is

relatively straightforward since transgenic technology can be used to introduce genes into animals and the transgenes will produce the desired protein without provoking an immune response.

5

Example 6

Selection of Subgroup A Avian Leukosis Virus Mutants Resistant to the Receptor

Interference Imposed by Soluble TVA and SUA

Genes encoding soluble forms of the subgroup A avian leukosis virus (ALV) receptor Tva (sTva) and soluble envelope surface glycoprotein (SUA), delivered by ALV-based retroviral vectors to cells, significantly inhibit ALV(A) infection of those cells. The antiviral effects of sTva and SUA are consistent with a receptor interference (RI) mechanism; the antiviral effect is specific for ALV(A) since the susceptibility to infection by a neutral subgroup remains unchanged. A virus-free stable cell line was developed which expressed either sTva or SUA and so was significantly resistant to ALV(A) infection, 50-350-fold and 1,000 to 200,000-fold, respectively. Since the SU region of Env determines receptor usage, mutations capable of overcoming the RI may occur in the SU region of the *env* gene of ALV(A) and would affect interactions between Tva and Env(A).

ALV(A) was passaged on the sTva and SUA expressing cell lines. Variant viruses capable of more rapid growth on the sTva cell lines were identified at passage 7 and on the SUA cell lines at passage 9 post-infection. The SU region of the *env* gene was PCR amplified from these cells, cloned and analyzed by sequencing. Selection on sTva expressing cells produced mutant viruses which were identical in sequence to ALV(A) except that 50% of the clones screened had an amino acid change at codon 142, while the other 50% had an amino acid change at codon 149 of *env*. All mutants selected on SUA expressing cell lines contained a 6 amino acid deletion at codons 155-160 within *env*. To determine if these mutations were sufficient to confer enhanced growth on the resistant cell lines, the SU region of *env* containing the mutation, from Kpn I to Sal I, was introduced into a wild-type ALV(A) molecular clone.

Transfection of these mutants showed an enhanced rate of growth as compared to wild-type.

5

References

- Achong, G. G., P. A. Trumper, and B. C. Giovanella. C-type virus particles in human tumors transplanted into nude mice. British Journal of Cancer, 34:203-206 (1976).
- 10 Adkins, H. B., J. Brojtsch, J. Naughton, M. M. Rolls, J. M. Pesola, and J.A.T. Young. Identification of a cellular receptor for subgroup E avian leukosis virus. PNAS. 94:11617-11622 (1997).
- Akiyoshi, E. E., M. Denaro, H. Zhu, J. L. Greenstein, P. Banerjee, and J. A. Fishman. Identification of a full-length cDNA for an endogenous retrovirus of miniature swine. Journal of Virology, 72:4503-4507 (1998).
- 15 Armstrong, J. A., J. S. Porterfield, and A. T. de Madrid. C-type virus particles in pig kidney cell lines. Journal of General Virology, 10:195-198 (1971).
- Astrin, S. M., E. G. Buss, and W. S. Hayward. Endogenous viral genes are non-essential in the chicken. Nature. 282:339-341 (1979).
- 20 Balliet, J. W., and P. Bates. Efficient infection mediated by viral receptors incorporated into retroviral particles. Journal of Virology. 72:671-676 (1998).
- Balliet, J. W., J. Berson, C. M., D'Cruz, J. Huang, J. Crane, J. M. Gilbert, and P. Bates. Production and characterization of a soluble, active form of TVA, the subgroup A avian sarcoma and leukosis virus receptor. Journal of Virology. 73:3054-3061 (1999).
- 25 Bassin, R. H., N. Tuttle, and J. R. Fischinger. Rapid cell culture assay technique for murine leukemia viruses. Nature (London), 229:564-566 (1971).
- 30 Bates, P., L. Rong, H. E. Varmus, J. A. T. Young, and L. B. Crittenden. Genetic mapping of the cloned subgroup A avian sarcoma and leukosis virus receptor gene to the TVA locus. Journal of Virology. 72:2505-2508 (1998).

Bates, P., J. A. T. Young, and H. E. Varmus. A receptor for subgroup A Rous sarcoma virus is related to the low density lipoprotein receptor. *Cell*. 74:1043-1051 (1993).

- Belanger, C., K. Zingler, and J. A. Young. Importance of cysteines in the
5 LDLR-related domain of the subgroup A avian leukosis and sarcoma virus
receptor for viral entry. *Journal of Virology*. 69:1019-1024 (1995).

- Bergelson, J. J., J. G. Mohanty, R. L. Crowell, N. F. St. John, D. M.
Lublin, and R. W. Finberg. Coxsackievirus B3 adapted to growth in RD cells
binds to decay-accelerating factor (CD55). *Journal of Virology*, 69:1903-1906
10 (1995).

Boeke, J. D., and J. P. Stoye. Retrotransposons, endogenous retroviruses,
and the evolution of retroelements. In J. M. Coffin and S. H. Hughes and H. E.
Varms (ed.), *Retroviruses*, p. 343-435, Cold Spring Harbor Laboratory Press.

- Bostock, D. E., and L. N. Owen. Porcine and ovine lymphosarcoma: A
15 review. *Journal of the National Cancer Institute*, 50:933-939 (1973).

Bova, C. A., J. P. Manfredi, and R. Swanstrom. Env genes of avian
retroviruses: nucleotide sequence and molecular recombinants define host range
determinants. *Virology*. 152:343-354 (1986).

- Bova, C. A., J. C. Olsen, and R. Swanstrom. The avian retrovirus env
20 gene family: molecular analysis of host range and antigenic variants. *Journal of
Virology*. 62:75-83 (1988).

- Breimer, M. E., S. Bjorck, S. C. T., A. Bengtsson, L. Rydberg, K. Lie-
Karlsen, P.-O. Attman, M. Aurell, and B. E. Samuelsson. Extracorporeal ("ex
vivo") connection of pig kidneys to humans. I. Clinical data and studies of
25 platelet destruction. *Xenotransplantation*, 3:328-339 (1996).

Brojatsch, J., J. Naughton, M. M. Rolls, K. Zingler, and J. A. T. Young.
CAR1, a TNFR-related protein, is a cellular receptor for cytopathic avian
leukosis-sarcoma viruses and mediates apoptosis. *Cell*. 82:845-855 (1996).

- Chong, H., W. Starkey, and R. G. Vile. A replication-competent
30 retrovirus arising from a split-function packaging cell line was generated by
recombination events between the vector, one of the packaging constructs, and
endogenous retroviral sequences. *Journal of Virology*, 72:2663-2670 (1998).

- Connolly, L., K. Zingler, and J. A. T. Young. A soluble form of a receptor for subgroup A avian leukosis and sarcoma viruses (ALSV-A) blocks infection and binds directly to ALSV-A. Journal of Virology, 68:2760-2764 (1994).
- 5 Crawford, D. H., B. G. Achong, N. M. Reich, S. Finerty, J. L. Thompson, M. A. Epstein, and B. C. Giovanella. Identification of murine endogenous xenotropic retrovirus in cultured multicellular tumor spheroids from nude-mouse-passaged nasopharyngeal carcinoma. International Journal of Cancer, 23:1-7 (1979).
- 10 Crittenden, L. B., D. W. Salter, and M. J. Federspiel. Segregation, viral phenotype, and proviral structure of 23 avian leukosis viruses inserted in the germ line of chickens. Theoretical Applied Genetics, 77:505-515 (1989).
- Daar, E. S., X. L. Li, T. Moudgil, and D. D. Ho. High concentrations of recombinant soluble CD4 are required to neutralize primary human
- 15 immunodeficiency virus type 1 isolates. Proceedings of the National Academy of Sciences, USA, 87:6574-6578 (1990).
- Damico, R., L. Rong, and P. Bates. Substitutions in the receptor-binding domain of the avian sarcoma and leukosis virus envelope uncouple receptor-triggered structural rearrangements in the surface and transmembrane subunits.
- 20 Journal of Virology, 73:3087-3094 (1999).
- Deacon, T., J. Schumacher, J. Dinsmore, C. Thomas, P. Palmer, S. Kott, A. Edge, D. Penney, S. Kassissieh, P. Dempsey, and O. Isacson. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. Nature Medicine, 3:350-353 (1997).
- 25 Diamond, L. E. K. R. McCurry, M. J. Martin, S. B. McClellan, E. R. Oldham, J. L. Platt, and J. S. Logan. Characterization of transgenic pigs expressing functionally active human CD59 on cardiac endothelium. Transplantation, 61:1241-1249 (1996).
- Donahue, R. E., S. W. Kessler, D. Bodine, K. McDonagh, C. Dunbar, S.
- 30 Goodman, B. Agricola, E. Byrne, M. Raffeld, R. Moen, J. Bacher, K. M. Zsebo, and A. W. Nienhuis. Helper virus induced T cell lymphoma in nonhuman primates after retroviral mediated gene transfer. The Journal of Experimental Medicine, 176:1125-1135 (1992).

- Dorig, R. E., A. Marcil, A. Chopra, and C. D. Richardson. The human CD46 molecule is a receptor for measles virus (Edmonston Strain). Cell, **75**:295-305 (1993).
- Ebert et al., Biotech, **9**, 835 (1991).
- 5 Federspiel, M. J., L. B. Crittenden, and S. H. Hughes. Expression of avian reticuloendotheliosis virus envelope confers host resistance. Virology, **173**:167-177 (1989).
- Federspiel, M. J., L. B. Crittenden, L. P. Provencher, and S. H. Hughes. Experimentally introduced defective endogenous proviruses are highly expressed
10 in chickens. Journal of Virology, **65**:313-319 (1991).
- Federspiel, M.J., and S.H. Hughes. Effects of the *gag* region on genome stability: avian retroviral vectors that contain sequences from the Bryan strain of Rous sarcoma virus. Virology, **203**:211-220 (1994).
- Federspiel, M.J., D.A. Swing, B. Eagleson, S.W. Reid, and S.H. Hughes.
15 Expression of transduced genes in mice generated by infecting blastocysts with avian leukosis virus-based retroviral vectors. Proc. Natl. Acad. Sci. USA, **93**:4931-4936 (1996).
- Federspiel, M. J., P. Bates, J. A. T. Young, and H. E. H. Varmus, S.H. A system for tissue-specific gene targeting: Transgenic mice susceptible to
20 subgroup A avian leukosis virus-based retroviral vectors. Proc. Natl. Acad. Sci. USA, **91**:11241-11245 (1994).
- Federspiel, M. J., and S. H. Hughes. Retroviral gene delivery. Methods in Cell Biology. **52**:179-214 (1997).
- Fekete, D. M., and C. L. Cepko. Retroviral infection coupled with tissue
25 transplantation limits gene transfer in the chicken embryo. Proc. Natl. Acad. Sci. USA. **90**:2350-2354 (1993).
- Fields-Berry, S. C., A. L. Halliday, and C. L. Cepko. A recombinant retrovirus encoding alkaline phosphatase confirms clonal boundary assignment in lineage analysis of murine retina. Proceedings of the National Academy of
30 Sciences, USA. **89**:693-697 (1992).
- Fishman, J. A., Miniature swine as organ donors for man: strategies for prevention of xenotransplant-associated infections. Xenotransplantation, **1**:47-57 (1994).

- Fodor, W. L., B. L. Williams, L. A. Matis, J. A. Madri, S. A. Rollins, J. W. Knights, W. Velander, and S. P. Squinto. Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneic hyperacute organ rejection. Proceedings of the National Academy of Sciences, 91:11153-11157 (1994).
- 5 Frazier, M. E. Evidence for retrovirus in miniature swine with radiation-induced leukemia or metaplasia. Archives of Virology, 83:83-97 (1985).
- Gautsch, J. W., A. F. Knowles, F. C. Jensen, and N. O. Kaplan. Highly efficient induction of type C retroviruses by a human tumor in athymic mice.
- 10 Proceedings of the National Academy of Sciences, 77:2247-2250 (1980).
- Gilbert, J. M., L. D. Hernandez, J. W. Balliet, P. Bates, and J. M. White. Receptor-induced conformational changes in the subgroup A avian leukosis and sarcoma virus envelope glycoprotein. Journal of Virology, 69:7410-7415 (1995).
- 15 Givol, I., I. Tsarfaty, J. Resau, S. Rulong, P. P. da Silva, G. Nasioulas, J. DuHadaway, S. H. Hughes, and D. L. Ewert. Bcl-2 expressed using a retroviral vector is localized primarily in the nuclear membrane and the endoplasmic reticulum of chicken embryo fibroblasts. Cell Growth and Differentiation, 5:419-429 (1994).
- 20 Groth, C. G., O. Korsgren, A. Tibell, J. Tollemar, E. Moller, J. Bolinder, J. Ostman, F. P. Reinholt, C. Hellerstrom, and A. Andersson. Transplantation of porcine fetal pancreas to diabetic patients. Lancet, 344:1402-1404 (1994).
- Harbison, M. A., J. M. Gillis, P. Pinkston, R. A. Byrn, R. M. Rose, and S. M. Hammer. Effects of recombinant soluble CD4 (rCD4) on HIV-1 infection
- 25 of monocyte/macrophages. Journal of Infectious Diseases, 161:1-6 (1990).
- Heneine, W., A. Tibell, W. M. Switzer, P. Sandstrom, G. Vazquez Rosales, A. Mathews, O. Korsgren, L. E. Chapman, T. M. Folks, and C. G. Groth. No evidence of infection with porcine endogenous retrovirus in recipients of porcine isle-cell xenografts. Lancet, 352:695-699 (1998).
- 30 Henninghausen, Protein Expression and Purification, 1:3 (1990).
- Hernandez, L. D., R. J. Peters, S. E. Delos, J. A. T. Young, D. A. Agard, and J. M. White. Activation of a retroviral membrane fusion protein: soluble

receptor-induced liposome binding of the ALSV envelope glycoprotein. Journal of Cell Biology. 139:1455-1464 (1997).

- 5 Himly, M., D. N. Foster, I. Bottoli, J. S. Iacovoni, and P. K. Vogt. The DF-1 chicken fibroblast cell line: transformation induced by diverse oncogenes and cell death resulting from infection by avian leukosis viruses. Virology. 248:295-304 (1998).

Hirsch, M. S., P. H. Black, G. S. Tracy, S. Leibowitz, and R. S. Schwartz. Leukemia virus activation in chronic allogeneic disease. Proceedings of the National Academy of Sciences, 67:1914-1917 (1970).

- 10 Hirsch, M. S., S. M. Phillips, C. Solnik, P.H. Black, R.S. Schwartz, and C.B. Carpenter. Activation of leukemia viruses by graft-versus-host and mixed lymphocyte reactions *in vitro*. Proceedings of the National Academy of Sciences, 69:1069-1072 (1972).

- Hughes, S. H., J. J. Greenhouse, C. J. Petropoulos, and P. Sutrawe.
15 Adaptor plasmids simplify the insertion of foreign DNA into helper-independent retroviral vectors. Journal of Virology. 61:3004-3012 (1987).

Hunter, E. Viral entry and receptors, p. 71-119. *In* J. M. Coffin and S. H. Hughes and H. E. Varmus (ed.), Retroviruses. Cold Spring Harbor Laboratory Press (1997).

- 20 Jaenish, Science, 240:468 (1988).

Kanaya, S., and R.J. Crouch. Low levels of RNase H activity in *Escherichia coli* FB2 *mh* result from a single-base change in the structural gene of RNase H. J. Bacteriol., 154:1021-1026 (1983).

- Kawakami, T.G., G.V. Kollias, C. Holmberg. Oncogenicity of Gibbon
25 type-C myelogenous leukemia virus. International Journal of Cancer, 25:641-646 (1980).

- Kingston, R. E., C. A. Chen, and H. Okayama. Introduction of DNA into eukaryotic cells, p. 911-919. *In* F. M. Ausubel and R. Brent and R. E. Kingston and D. D. Moore and J. G. Seidman and J. A. Smith and K. Struhl (ed.), Current
30 Protocols in Molecular Biology, vol. 1. Wiley, New York (1989).

Klasse, P. J., and J. A. McKeating. Soluble CD4 and CD4 immunoglobulin-selected HIV-1 variants: a phenotypic characterization. AIDS Research and Human Retroviruses. 9:595-604 (1993).

- Le Tissier, P., J.P. Stoye, Y. Takeuchi, C. Patience, and R.A. Weiss. Two sets of human-tropic pig retrovirus. Nature, 389:681-682 (1997).
- Leverett, B.D., K.B. Farrell, M.V. Eiden, and C.A. Wilson. Entry of amphotropic murine leukemia virus is influenced by residues in the putative second extracellular domain of the human form of its receptor, Pit2, L.Virol., 72, (6) 4956-4961 (1998).
- Levy, J.A., S.K. Datta, and R.S. Schwartz. Recovery of xenotropic virus but not ecotropic virus during graft-versus-host reaction in mice. Clinical Immunology and Immunopathology, 7:262-268 (1977).
- 10 Lieber, M.M., C.J. Sherr, G.J. Todaro, R.E. Benveniste, R. Callahan, and H.G. Coon. Isolation from the Asian mouse *Mus caroli* of an endogenous type C virus related to infectious primate type C viruses. Proceedings of the National Academy of Sciences, 72:2315-2319 (1975).
- Lin, S.S., and J.L. Platt. Immunologic barriers to xenotransplantation. 15 The Journal of Heart and Lung Transplantation, 15:547-555 (1996).
- Makowka, L., D.V. Cramer, A. Hoffman, M. Breda, L. Sher, G. Eiras-Hrehla, P.J. Tuso, C. Yasunaga, C.A. Cosenza, G. Du Wu, F.A. Chapman, and L. Podesta. The use of a pig liver xenograft for temporary support of a patient with fulminant hepatic failure. Transplantation, 59:1654-1659 (1995).
- 20 Marshall et al., Cell & Mol. Neuro., 14, 523 (1994).
- Martin, U., V. Kiessig, J.H. Blusch, A. Haverich, K. von der Helm, T. Herden, and G. Steinhoff. Expression of pig endogenous retrovirus by primary porcine endothelial cell and infection of human cells. Lancet, 352:692-694 (1998).
- 25 McCurry, K.R., D.L. Kooyman, C.G. Alvarado, A.H. Cotterell, M.J. Martin, J.S. Logan, and J.L. Platt. Human complement regulatory proteins protect swine-to-primate cardiac xenografts from humoral injury. Nature Medicine. 1:423-427 (1995).
- McLachlin, J.R., N. Mittereder, M.B. Daucer, M. Kadan, and M.A. 30 Eglitis. Factors affecting retroviral vector function and structural integrity. Virology, 195:1-5 (1993).
- Moennig, V., H. Frank, G. Hunsmann, P. Ohms, H. Schwarz, W. Schafer, and H. Strandstrom. C-type particles produced by a permanent cell line from a

leukemic pig II. Physical, chemical, and serological characterization of the particles. Virology, 57:179-188 (1974).

- Morgan, R. A., G. Baler-Bitterlich, J. A. Ragheb, F. Wong-Staal, R. C. Gallo, and W. F. Anderson. Further evaluation of soluble CD4 as an anti-HIV
5 type 1 gene therapy: demonstration of protection of primary human peripheral
blood lymphocytes from infection by HIV type 1. AIDS Research and Human
Retroviruses. 10:1507-1515 (1994).

Natsoulis, G., and J.D. Boeckle. New antiviral strategy using capsid-
nuclease fusion proteins. Nature (London), 352:632-635 (1991).

- 10 Natsoulis, G., P. Seshiah, M.J. Federspiel, A. Rein, S.H. Hughes, and
J.D. Boeckle. Targeting a nuclease to murine leukemia virus capsids inhibits viral
multiplication. Proceedings of the National Academy of Sciences, USA,
92:364-368 (1995).

- Nihrane, A., K. Fujita, R. Willey, M. S. Lyu, and J. Silver. Murine
15 leukemia virus envelope protein in transgenic-mouse serum blocks infection *in
vitro*. Journal of Virology, 70:1882-9 (1996).

- Oldham, E.R., M.A. Velardo, J.L. Platt, J.S. Logan, and L. Diamond.
High-level tissue specific expression of human CD59, MCP, and DAF proteins
from genomic clones in transgenic mice. Transplantation Proceedings, 28:693
20 (1996).

- Orloff, S. L., M. S. Kennedy, A. A. Belperron, P. J. Maddon, and J. S.
McDougal. Two mechanisms of soluble CD4 (sCD4)-mediated inhibition of
human immunodeficiency virus type 1 (HIV-1) infectivity and their relation to
primary HIV-1 isolated with reduced sensitivity to sCD4. Journal of Virology.
25 67:1461-1471 (1993).

Ott, D., R. Fredrich, and A. Rein. Sequence analysis of amphotropic and
10A1 murine leukemia viruses: close relationship to mink cell focus-inducing
viruses. J. Virol., 64:757-766 (1990).

- Ott, D.E., J. Keller, K. Sill, and A. Rein. Phenotypes of murine leukemia
30 virus-induced tumors: influence of 3' viral coding sequences. J. Virol., 66:6107-
6116 (1992).

Panabieres, F., M. Piechaczyk, B. Rainer, C. Dani, P. Fort, S. Riaad, L.
Marty, J. L. Imbach P. Jeanteur, and J. Blanchard. Complete nucleotide

sequence of the messenger RNA coding for chicken muscle glyceraldehyde-3-phosphate dehydrogenase. Biochem. Biophys. Res. Comm. 118:767-773 (1984).

- Patience, C., G.S. Patton, Y. Takeuchi, R.A. Weiss, M.O. McClure, L. Rydberg, and M.E. Breimer. No evidence of pig DNA or retroviral infection in patients with short-term extracorporeal connection to pig kidneys. Lancet, 352:699-701 (1998).

- Patience, C., G.R. Simpson, A.A. Colletta, H.M. Welch, R.A. Weiss, and M.T. Boyd. Human endogenous retrovirus expression and reverse transcriptase activity in the T47D mammary carcinoma cell line. Journal of Virology, 70:2654-2657 (1996).

Patience, C., Y. Takeuchi, and R.A. Weiss. Infection of human cells by an endogenous retrovirus of pigs. Nature Medicine, 3:282-286 (1997).

- Petropoulos, C. J., and S. H. Hughes. Replication-competent retrovirus vectors for the transfer and expression of gene cassettes in avian cells. Journal of Virology. 65:3728-3737 (1991).

Petropoulos, C. J., W. Payne, D. W. Salter, and S. H. Hughes. Appropriate *in vivo* expression of a muscle-specific promoter by using avian retroviral vectors for gene transfer. Journal of Virology. 66:3391-3397 (1992).

- Phan-Thanh, L., B. Kaeffer, and E. Bottreau. Porcine retrovirus: optimal conditions for its biochemical detection. Archives of Virology, 123:255-265 (1992).

Policy, C.o.X.T.E.I.a.P. Xenotransplantation science, ethics, and public policy. Institute of Medicine (1996).

- Purcell, D.F., C.M. Broscious, E.F. Vanin, C.E. Buckler, A.W. Nienhuis, and M.A. Martin. An array of murine leukemia virus-related elements is transmitted and expressed in a primate recipient of retroviral gene transfer. Journal of Virology, 70:887-897 (1996).

- Ramsdell, F.J., H. Shau, and S.H. Golub. Role of proliferation in LAK cell development, Cancer Immunol. Immunother., 26, 139-144 (1988).

Robinson, H. L., S. M. Astrin, A. M. Senior, and F. H. Salazar. Host susceptibility to endogenous viruses: defective, glycoprotein-expressing proviruses interfere with infections. Journal of Virology. 40:745-751 (1981).

Rogers et al. *PNAS*, 90:5777 (1993).

Rong, L., and P. Bates. Analysis of the subgroup A avian sarcoma and leukosis virus receptor: the 40-residue, cysteine-rich, low-density lipoprotein receptor repeat motif of Tva is sufficient to mediate viral entry. *Journal of Virology*, 69:4847-4853 (1995).

Rong, L., K. Gendron, and P. Bates. Conversion of a human low-density lipoprotein receptor ligand-binding repeat to a virus receptor: identification of residues important for ligand specificity. *PNAS*, 95:8467-8472 (1998).

10 Rosenberg, N., and P. Jolicoeur. Retroviral pathogenesis, p. 475-585. In J.M. Coffin and S.H. Hughes and H.E. Varmus (ed.), *Retroviruses*, Cold Spring Harbor Laboratory Press (1997).

Ryan, U.S. Complement inhibitory therapeutics and xenotransplantation. *Nature Medicine*, 1:967-968 (1995).

15 Salter, D. W., E. J. Smith, S. H. Hughes, S. E. Wright, and L. B. Crittenden. Transgenic chickens: Insertion of retroviral genes into the chicken germline. *Virology*, 157:236-240 (1987).

Salter, D. W., and L. B. Crittenden. Artificial insertion of a dominant gene for resistance to avian leukosis virus into the germ line of the chicken. *Theoretical Applied Genetics*, 77:457-461 (1989).

20

Salter, D. W., and L. B. Crittenden. Insertion of a disease resistance gene into the chicken germline. *Biotechnology*, 16:125-131 (1991).

Sambrook, J., E.F. Fritsch, and T. Maniatis. *Molecular cloning: a laboratory manual*, Second edition ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

25

Sandrin, M.S., W.L. Fodor, E. Mouhtouris, N. Osman, S. Cohny, S.A. Rollins, E.R. Guilmette, E. Setter, S.P. Squinto, and I.F.C. McKenzie. Enzymatic remodeling of the carbohydrate surface of a xenogeneic cell substantially reduces human antibody binding and complement-mediated cytotoxicity. *Nature Medicine*, 1:1261-1267 (1995).

30

Schacker, T., A. C. Collier, R. Coombs, J. D. Unadkat, I. Fox, J. Alam, J.-P. Wang, E. Eggert, and L. Corey. Phase I study of high-dose, intravenous

rsCD4 in subjects with advanced HIV-1 infection. Journal of Acquired Immune Deficiency Syndromes. 9:145-152 (1995).

Schaefer-Klein, J., I. Givol, E. V. Barsov, J. M. Whitcomb, M.

VanBrocklin, D. N. Foster, M. J. Federspiel, and S. H. Hughes. The EV-0-

- 5 derived cell line DF-1 supports efficient replication of avian leukosis-sarcoma viruses and vectors. Virology. 248:305-311 (1998).

Schumann, G., L. Qin, A. Rein, G. Natsoulis, and J.D. Boeckle.

Therapeutic Effect of *Gag*-nuclease fusion protein on retrovirus-infected cell cultures. J. Virol., 70:4329-4339 (1996).

- 10 Seifarth, W., C. Baust, A. Murr, H. Skladny, F. Krieg-Schneider, J.

Blusch, T. Werner, R. Hehlmann, and C. Mosch. Proviral structure, chromosomal location, and expression of HERV-K-T47D, a novel human endogenous retrovirus derived from T47D particles. Journal of Virology, 72:8384-8391 (1998).

- 15 Sherr, C.J., L.A. Fedele, R.E. Benveniste, and G.J. Todaro. Interspecies antigenic determinants of the reverse transcriptases and p30 proteins of mammalian type C viruses. Journal of Virology. 15:1440-1448 (1975).

Sherr, C.J. M.M. Lieber, and G.J. Todaro. Mixed splenocyte cultures and graft versus host reactions selectively induce an "S-tropic" murine type C virus.

- 20 Cell, 1:55-58 (1974).

Sielaff, T.D., M.Y. Hu, S. Rao, K. Groehler, D. Olson, H.J. Mann, R.P.

Rommel, R.A.D. Shatford, B. Amiot, W.-S. Hu, and F.B. Cerra. A technique for porcine hepatocyte harvest and description of differentiated metabolic functions in static culture. Transplantation, 59:1459-1463 (1995).

- 25 Simpson, G.R., C. Patience, R. Lower, R.R. Tonjes, H.D.M. Moore, R.A. Weiss, and M.T. Boyd. Endogenous D-type (HERV-K) related sequences are packaged into retroviral particles in the placenta and possess open reading frames for reverse transcriptase. Virology, 222:451-456 (1996).

Smith, E. J., J. Brojatsch, J. Naughton, and J. A. T. Young. The CARI

- 30 gene encoding a cellular receptor specific for the subgroup B and D avian leukosis viruses maps to the chicken tvb locus. Journal of Virology. 72:3501-3503 (1998).

- Smith, E. J., A. M. Fadly, and W. Okazaki. An enzyme-linked immunoabsorbant assay for detecting avian leukosis sarcoma viruses. Avian Diseases, 23:698-707 (1979).
- Strandstrom, H., P. Veijalainen, V. Moening, G. Hunsmann, H. Schwarz,
5 and W. Schafer. C-type particles produced by a permanent cell line from a leukemia pig I. Origin and properties of the host cells and some evidence for the occurrence of c-type-like particles. Virology, 57:175-178 (1974).
- Suzuka, I., K. Sekiguchi, and M. Kodama. Some characteristics of a porcine retrovirus from a cell line derived from swine malignant lymphomas.
10 FEBS Letters, 183:124-128 (1985).
- Suzuka, I., N. Shimizu, K. Sekiguchi, H. Hoshino, M. Kodama, and K. Shimotohno. Molecular cloning of unintegrated closed circular DNA of porcine retrovirus. FEBS Letters, 198:339-343 (1986).
- Suzuki, T., K. Yanagihara, K. Yoshida, T. Seido, N. Kuga, Y. Shimosato,
15 and S. Oboshi. Infectious murine type-c viruses released from human cancer cells transplanted into nude mice. Cancer, 68:96-106 (1977).
- Takeuchi, Y., C. Patience, S. Magre, R.A. Weiss, P.T. Banerjee, P. LeTissier, and J.P. Stoye. Host range and interference studies of three classes of pig endogenous retrovirus. Journal of Virology, 72:9986-9991 (1998).
- 20 Todaro, G.J., R.E. Benveniste, M.M. Lieber, and C.J. Sherr. Characterization of a type C virus released from the porcine cell line PK (15). Virology, 58:65-74 (1974).
- Tralka, T.S., C.L. Yee, A.B. Rabson, N.A. Wivel, K.J. Stromberg, A.S. Rabson, and J.C. Costa. Murine type C retroviruses and intracisternal A-
25 particles in human tumors serially passaged in nude mice. Journal of the National Cancer Institute, 71:591-599 (1983).
- Tucker, P. W., K. B. Marcu, J. L. Slightom, and F. R. Blattner. Structure of the cloned gene for the constant region of murine gamma 2b immunoglobulin heavy chain. Science. 206:1299-1306 (1979).
- 30 VanBrocklin, M., A.L. Ferris, S.H. Hughes, and M.J. Federspiel. Expression of a murine leukemia virus Gag-Escherichia coli RNase HI fusion polyprotein significantly inhibits virus spread. Journal of Virology, 71:3312-3318 (1997).

Vanin, E.F., M. Kaloss, C. Broscius, and A.W. Nienhuis.

Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis. Journal of Virology, 68:4241-4250 (1994).

- 5 Ward, T., P.A. Pipkin, N.A. Clarkson, D.M. Stone, P.D. Minor, and J.W. Almond. Decay-accelerating factors CD55 is identified as the receptor for echovirus 7 using CELICS, a rapid immuno-focal cloning method. The EMBO Journal, 13:5070-5074 (1994).

- 10 Weiss, R.A. Transgenic pigs and virus adaptation. Nature, 391:327-328 (1998).

Weiss, R. Experimental biology and assay of RNA tumor viruses, p. 209-260. In R. Weiss and N. Teich and H. Varmus and J. Coffin (ed.), RNA Tumor Viruses, vol. 1. Cold Spring Harbor Laboratory Press, New York (1982).

- 15 Weiss, R. A. Cellular receptors and viral glycoproteins involved in retrovirus entry, p. 1-108. In J. A. Levy (ed.), The Retroviruses, vol. 2. Plenum Press, New York (1992).

Wilson, C., and M. Eiden. Viral and cellular factors governing hamster cell infection by murine and gibbon ape leukemia viruses, J. Virol., 65(11) 5975-5982 (1991).

- 20 Wilson, C.A., M.V. Eiden, and J.W. Marsh. Quantitative micro P30 and reverse transcriptase assays for Moloney murine leukemia virus. Journal of Virology Methods, 48:109-118 (1994).

- 25 Wilson, C.A., S. Wong, J. Muller, C.E. Davidson, T.M. Rose and P. Burd. Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. Journal of Virology, 72:3082-3087 (1998).

Wright et al., Biotech 2:830 (1991).

- 30 Wu, X., H. Liu, H. Xiao, J. A. Conway, and J. C. Kappes. Inhibition of human and simian immunodeficiency virus protease function by targeting Vpx-protease-mutation fusion protein into viral particles. J. Virol., 10:3378-3384 (1996).

Wunderli, H., D.D. Mickey, and D.F. Paulson. C-type virus particles in human urogenital tumors after heterotransplantation into nude mice. British Journal of Cancer, 39:35-42 (1979).

- Yodoi, J.K., Teshigawara, T. Nikaido, F. Fukui, T. Noma, T. Hongo, M. Takigawa, M. Sasaki, N. Minato, M. Tsudo, T. Uchiyama, AND M. Maeda. 5 TCGR (IL-2)-receptor inducing factor(s). I. Regulation of IL-2 receptor on natural killer-like cell line (YT cells). J. Immunol., 134, 1623-1630 (1985).

- Young, J. A. T., P. Bates, and H. E. Varmus. Isolation of a chicken gene that confers susceptibility to infection by subgroup A avian leukosis and sarcoma 10 viruses. Journal of Virology. 67:1811-1816 (1993).

- All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled 15 in the art that the invention is susceptible to additional embodiments and that certain of the detailed herein may be varied considerably without departing from the basic principles of the invention.

WHAT IS CLAIMED IS:

1. A method to inhibit or prevent infectious agent transmission in a mammalian transplant recipient, comprising:
 - 5 a) introducing to donor swine cells a recombinant DNA comprising a promoter operably linked to a DNA segment encoding a protein comprising at least a portion of a polypeptide of the infectious agent that is present in the extracellular form of the agent so as to yield transformed swine cells; and
 - 10 b) introducing the transformed swine cells to the recipient.
2. A method to inhibit or prevent infectious agent transmission in a mammalian transplant recipient, comprising:
 - 15 a) introducing to donor human blood cells a recombinant DNA comprising a promoter operably linked to a DNA segment encoding a protein comprising at least a portion of a polypeptide of the infectious agent that is present in the extracellular form of the agent so as to yield transformed human blood cells; and
 - 20 b) introducing the transformed human blood cells to the recipient.
3. A method to inhibit or prevent infectious agent transmission to a mammalian transplant recipient, comprising:
 - 25 a) introducing to a donor organ a recombinant DNA comprising a promoter operably linked to a DNA segment encoding a protein comprising at least a portion of a polypeptide of the infectious agent that is present in the extracellular form of the agent so as to yield a transformed organ; and
 - b) introducing the transformed organ to the recipient.
- 30 4. The method of claim 1, 2 or 3 wherein the DNA segment encodes a fusion protein comprising at least a portion of a polypeptide of the infectious agent that is present in the extracellular form of the agent and a degradative enzyme.

5. The method of claim 4 wherein the degradative enzyme is a nuclease or protease.
6. The method of claim 1, 2 or 3 wherein the infectious agent is a virus.
- 5 7. The method of claim 6 wherein the polypeptide of the infectious agent is a viral capsid protein, viral glycoprotein or an accessory protein.
8. The method of claim 6 wherein the virus is a lentivirus, retrovirus, hepatitis virus or a herpesvirus.
- 10 9. The method of claim 1 wherein the donor cells are embryonic stem cells, blood cells, neuronal cells, liver cells, pancreatic cells, kidney cells or islet cells.
- 15 10. The method of claim 3 wherein the organ is a heart, liver or kidney.
11. The method of claim 3 wherein the organ is a human or pig organ.
- 20 12. The method of claim 4 wherein the DNA segment encodes a fusion protein encoding a polypeptide of a pig endogenous retrovirus.
13. The method of claim 5 wherein the enzyme is barnase, staphylococcal nuclease, RNase H1, RNase T1, retroviral protease, RNase III, RNaseL, or a ribozyme.
- 25 14. The method of claim 7 wherein the polypeptide of the infectious agent is Vpr, Vpx, Vif or Nef.
- 30 15. An isolated and purified nucleic acid molecule comprising a nucleic acid segment which comprises at least a portion of a pig endogenous retrovirus, wherein the nucleic acid segment hybridizes under hybridizing

conditions to SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:32.

16. An isolated and purified polypeptide encoded by a nucleic acid molecule comprising a nucleic acid segment comprising SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:32.
17. A method to detect human tropic pig endogenous retroviruses, comprising:
 - a) contacting a mammalian sample suspected of being infected with a pig endogenous retrovirus with a probe comprising at least a portion of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:32 so as to form complexes; and
 - b) detecting or determining the presence of the complexes.
18. A method of using an isolated and purified nucleic acid molecule comprising the genome of a pig endogenous retrovirus comprising:
 - introducing to a host cell a recombinant DNA molecule comprising a promoter operably linked to a DNA segment comprising SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, or SEQ ID NO:32 so as to yield a transformed host cell, and identifying the transformed host cell.
19. The method of claim 1, 2, 3 or 18 wherein the recombinant DNA molecule further comprises transcriptional termination sequences 3' to the DNA segment.
20. A host cell, the genome of which is augmented with a recombinant DNA molecule comprising a promoter operably linked to a DNA segment encoding a fusion protein comprising at least a portion of a polypeptide of a pig endogenous retrovirus and a degradative enzyme.

21. A fusion protein, comprising: a capsid or envelope protein of a porcine endogenous virus and a degradative enzyme.
22. The fusion protein of claim 21 wherein the enzyme is a nuclease.
- 5 23. The fusion protein of claim 21 wherein the enzyme is a protease.
24. The fusion protein of claim 21 wherein the enzyme is a lipase.
- 10 25. The fusion protein of claim 21 wherein the activity of said enzyme is calcium-dependent.
26. The fusion protein of claim 25 wherein the enzyme is staphylococcal nuclease.
- 15 27. An isolated and purified DNA molecule encoding the fusion protein of claim 21.
28. A recombinant virus comprising a nucleic acid molecule encoding the fusion protein of claim 21.
- 20 29. An antibody that specifically binds pig endogenous retrovirus.
30. The antibody of claim 29 which binds a viral capsid protein.
- 25 31. The antibody of claim 29 which binds the viral envelope glycoprotein.
32. The method of claim 8 wherein the virus is Epstein Barr virus.
- 30 33. The method of claim 8 wherein the virus is cytomegalovirus.
34. The method of claim 8 wherein the virus is human immunodeficiency virus.

35. The method of claim 1, 2, 3 or 18 wherein the recombinant DNA is introduced to the recipient by infection with a recombinant virus.
36. A method to inhibit or prevent infectious agent transmission in a mammalian transplant recipient, comprising:
- 5 a) introducing to donor mammalian cells a recombinant DNA comprising a promoter operably linked to a DNA segment encoding a protein comprising at least a portion of a polypeptide of the infectious agent that is present in the extracellular form of the agent so as to yield transformed mammalian cells; and
- 10 b) introducing the transformed mammalian cells to the recipient.
37. A method to inhibit or prevent infectious agent transmission in a mammalian transplant recipient, comprising:
- 15 a) introducing to donor human cells a recombinant DNA encoding a protein comprising at least a portion of a polypeptide of the infectious agent that is present in the extracellular form of the agent so as to yield transformed human cells; and
- 20 b) introducing the transformed human cells to the recipient.
38. The method of claim 36 or 37 wherein the infectious agent is a virus.
39. The method of claim 38 wherein the polypeptide of the infectious agent is a viral capsid protein, viral glycoprotein or an accessory protein.
- 25

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71726 A1

(51) International Patent Classification: C12N 15/48,
15/62, C07K 14/15, 19/00, A61K 48/00, C12Q 1/68,
C07K 16/10, C12N 7/01

(21) International Application Number: PCT/US00/14296

(22) International Filing Date: 24 May 2000 (24.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/135,631 24 May 1999 (24.05.1999) US

(71) Applicant (for all designated States except US): MAYO
MEDICAL VENTURES [US/US]; 200 First Street SW,
Rochester, MN 55905 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): FEDERSPIEL,
Mark, J. [US/US]; 5722 Colonial Lane SE, Rochester,
MN 55904 (US).

(74) Agent: VIKSNINS, Ann, S.; Schwegman, Lundberg,
Woessner & Kluth, P.O. Box 2938, Minneapolis, MN
55402 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE,
DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU,
ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,
LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS TO INHIBIT INFECTIOUS AGENT TRANSMISSION DURING XENOTRANSPLANTATION

(57) Abstract: The invention provides nucleic acid molecules, compositions and methods to inhibit or prevent infectious agent transmission from donor to recipient or recipient to donor during or after transplant.

WO 00/71726 A1

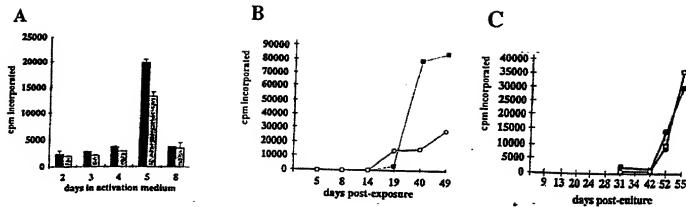


Fig 1

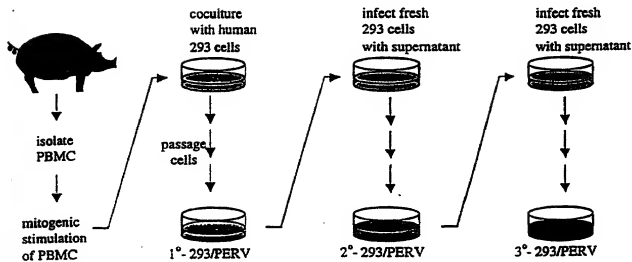


Fig 2

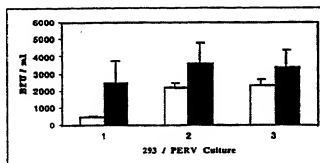


Fig 3

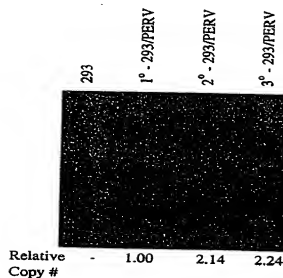


Fig 4

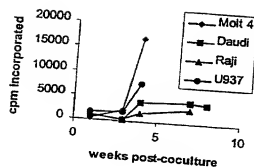


Fig 5

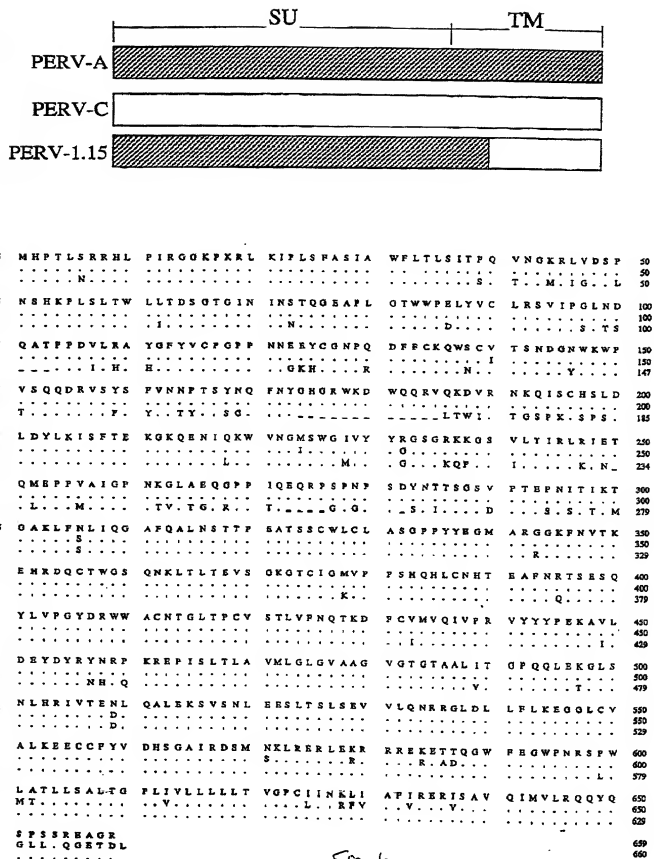


Fig. 6

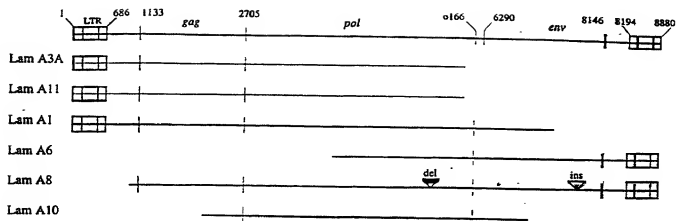


Fig. 7

Lens1	PERV-MSL	AATGAAAGGA	TGAAAATGCA	ACCTGACTCT	CCCAGAACC	AGGAAGTTAA	50
Lens1	PERV-MSL	TAAAGAGCTC	TAAATGCCCT	CGAATTCCAG	ACCTGTGTCC	CTATAGGTAA	100
Lens1	PERV-MSL	AAGATCATAC	TTTTTGGTGT	TTTAAATAT	GCTTCTGTCT	CTGTACAAAA	150
Lens1	PERV-MSL	CTTGTGGAA	GGGAAAAAAC	AGGCCCTTGA	GTATGTGCTT	CTATGCTTGA	200
Lens1	PERV-MSL	AACTTCTTGA	AACTGCTCT	AACTGCTTGT	TTGGCTTCTG	TAAACCTGCT	250
Lens1	PERV-MSL	TGCATAAGAT	AAAAAGAGGA	GAAATCAATT	GCCTAACGGA	CCCCAGTAA	300
Lens1	PERV-MSL	ATCGGTTGTA	CCACAAAAAT	TTGAAACACA	TATCTTGGTG	ACAACATGTC	350
Lens1	PERV-MSL	TCCCCACCC	CGAAACATGC	GCAAAATGT	AAGCTTAAAA	CAATTTAAAT	400
Lens1	PERV-MSL	TAATTGGTCC	ACGAAGCGCG	GGCTCTCGAA	GTTTTAAATT	GACTGGTTTG	450
Lens1	PERV-MSL	TGATATTTT	AAATGATTGG	TTTGTAAAGC	GCCTGCTTTG	TTGTGAACCC	500
Lens1	PERV-MSL	CATAAAAGCT	GTCCCGACTC	CACACTCGGG	GCCGCACTCC	TCTACCCCTG	550
Lens1	PERV-MSL	CGTGGTGTAC	GACTGTGGGC	CCCAGCGCGC	TTGGAATAAA	AATCCTCTTG	600
Lens1	PERV-MSL	CTGTTTGCAT	CAAGACCGCT	TCTCGTGAGT	GATTAAAGGG	AGTCGCCCTT	650
Lens1	PERV-MSL	TCCGAGCCTG	GAGGTTCTTT	TTGCTAGTCT	TACATTGGG	GGCTCGTCCG	700
Lens1	PERV-MSL	GGAT					750
Lens1	PERV-MSL						800

Fig. 8

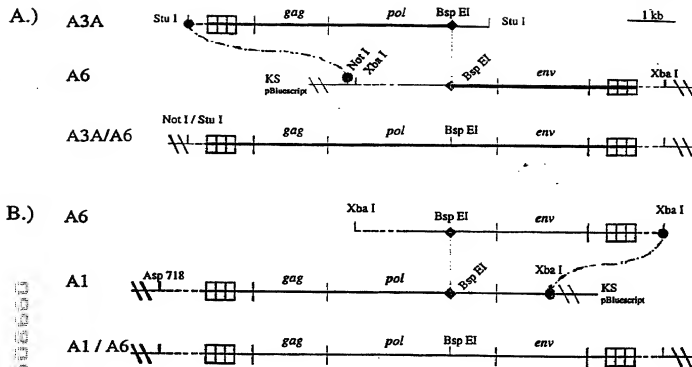


Fig 9

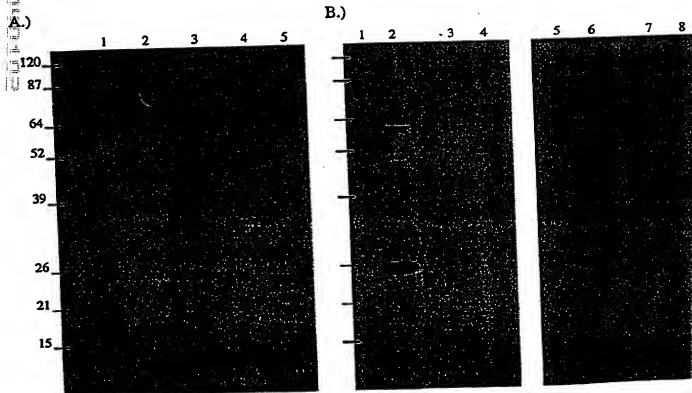


Fig. 10

REPLICATION COMPETENT VECTOR

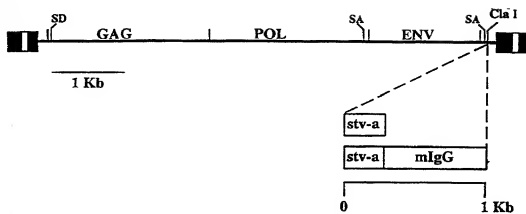


Figure 11

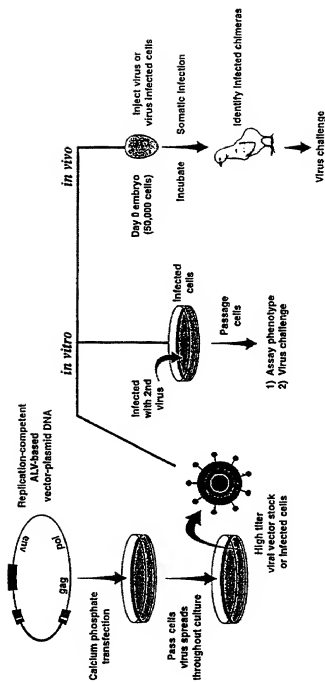


Figure 12

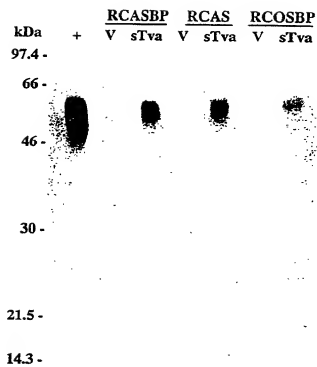


Figure 13

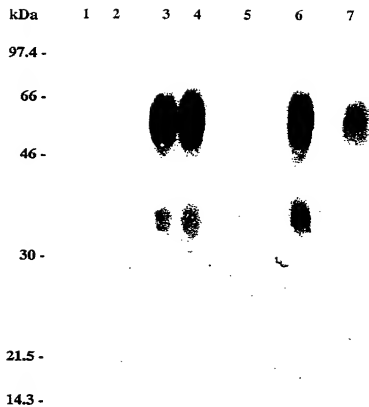


Figure 1a

2010-05-25 09:00

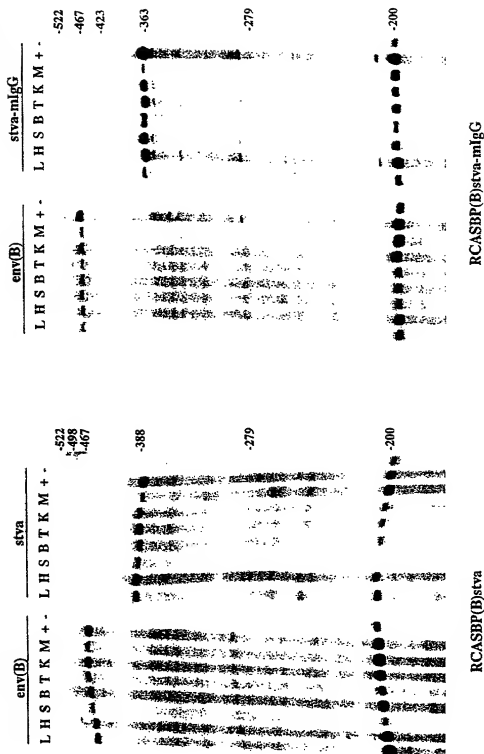
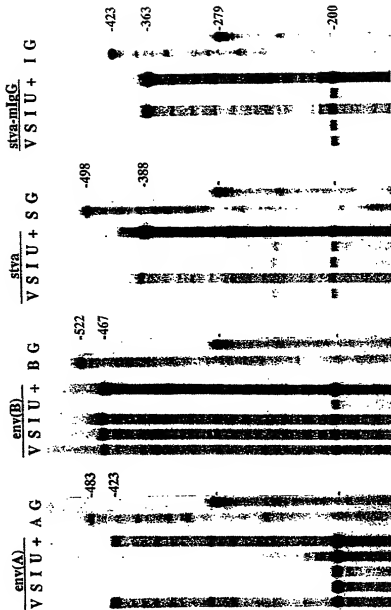


Figure 15

00980526 040100



PCIA 1/16

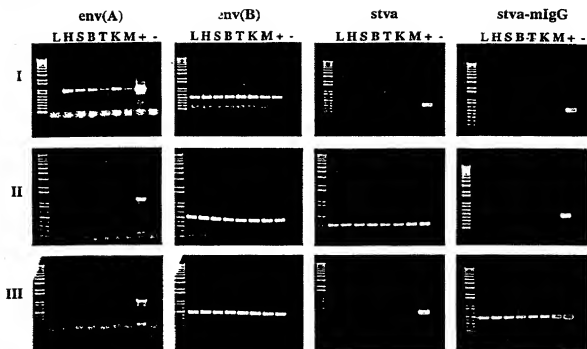


Figure 17

Figure 18

PERV 1.15

ATGCTATCCCAAGTTAAGCCGGCCGACCTCCGCGATTCCGGGTGGAAAGCCGAAGAAGCTCGAAATATCCCTTTAAGCTTCGCT
 CTCCTACCGCTGGTGGCTTACTCTGTCATAAATCTCTCAAGTTAAGTTGAACGCCCTTGTGGACAGCCGCAACTCCCATTA
 AACCCTTATCTCTACCTGTTTACTCTGACTCCGGTACAGGTATTAAATTAACAGCAGCTCAAGGAGGAGCGCTCCCTGT
 GCGACCTGTGGTGGTGAATATATGTTCTGCTTCCATCAGTAATCCCTGGTTCTCATTTGAACGAGGCCACACCCCGCATGT
 ACTCCGCTTACCGGCTTTTACGTTTTCGCCACGGGCCCAATAATGGAAGATATGTTGCAAAATCTCCAGGATATCTTCT
 CGAAGCAACTGGAGCTGGCTTAATCTTAATGATGGGAATGGAAATGGCCAGTCTCTCAGCAAGACAGAGTAAGTTTACTCT
 TTGTTTAAACAATCTCAACGTTATAATCAATTTAATTATGGCCATGGGAGATGAAAGATTGGCAACAGCGGTGTAACAAA
 AGATGTACGAAATAAAGCAATAAGCTGTCACTCGTACAGCTAGATTACTTAAATAAAGTTTCACTGCAAAAAAGGAAAAAC
 AAGAAAATATTCAAAGTATAGTATATGTTATGTTCTTCCGGAATAGTACTAGTACAGGGCTCTGGGAGAGGAAGAGATCT
 GTTCTGACTATTGCTCAGAAATGAAACTCAGATGGAACTCCGGTGTATAGGACCAAAATAGGTTTGGCGCAACA
 AGGACCTCCAATCCGAAGACAGAGGCCATCTCTAACCCCTTGATTACAATACAACTCTGGATCACTGCCACCTGAGC
 TTAACACTCACTATTAAACAGGGGCCAAACTTTTTAACTCTATCCAGGAGCTTTTCAAGCTTTAACTCCAGCAGTCCA
 GAGGCTTACTCTTCTTGTGGCTTGTGTAAGCTTCGGGCCACCTTACTATAGGGGAATGGCTAGAGGAGGAGAAATCCA
 GTGGGACAGGAAGAGCTGCCCTTATCTAGCACCCGCAACAGCTGGAGAAAGGAAGCTTACTAGCTTACTGAGGTTTGGAAAGGGCA
 CTTGCATAGGAGATGGTTCGCCCATCCCAACCACTTTGTAAACCACTGAAGCCCTTAAATGCAACCTCTGGAGGTGAG
 TATCTGGTACCTGGTTATGACAGGTGGTGGGCAATGTAATCTGGATTAAACCCCTTGTGTTTCCACCTTGTGTTTGAACCA
 AACTAAAGACTTTTGGCTTATGTTCCAAATTTGTCGCCGGGTGATCTACTATCCCGAAAAAGCAGCTCTGGATGTAAGT
 ACTATAGATATAATGGGCCAAAAAGAGAGCCCATATCTCTGACACTAGCTGTAAATGCTCGGATGGGAGTGGCTCGCAGGC
 GTGGGACAGGAAGAGCTGCCCTTATCTAGCACCCGCAACAGCTGGAGAAAGGAAGCTTACTAGCTTACTGAGTGAATGTAAC
 GGAATAATCCAGAGCTTGAAAAAATCTGTCAAGCTTGAGGAATCCCTAACCTCTTATCTGAAGTGGTTCACAGA
 ACAGAAAGGGGGTTAGATTGTATTCTAAAAAGGAGGAGTATGTTGTAACCTTAAAGGGGAGGAGCTGTTTATTGTG
 GATCATCTACGGGGCCATCAGAGACTCATGAAACAAGCTTAGAGAAAGTTGGAGAACGCTGCAAGGGAAGAAAGGAATAC
 TCAAGGGTGGTTTGGAGGATGGTTCAACAGGTCTCTGGTTGGTCACTCTCTTCTGCTCTTAAACAGGACCCCTTAATAG
 TCTCTCTCTGTGTCTACACAGTTGGGCCATGATATTAATAAGTAAATGCTCTTATTAAGAACGAATAAGTAGGATG
 CAGATCTGTTACTTAGACACAGATACCAAGGCCCTGTACTAGAGAAAGCTGGCCCTAG

Lama1

TACTCTTGGGGAAGACCTTGGGCTGCTAACTGGTGTCTGGCTGGTCTGATGAAAGGATGAAATGCCAACCTGACTCTC
 CACGAACCTCAAGGAGTTAATAAAGCTCTAAATAATGAAGGATGAAATGCAACCTGCAAGCCAGCCAGGAGAG
 TTAATAAGAAAGCTCTAAATGCCCTCGAATTCACAGACCTGTTCCCTATAGGTGAATAAGATCATACTTTTGTGTTTAGG
 CGCTGCTTCTTGCTGCTTGTACAAAACCTTTGTGGAAGGGGAAAAACAGGCCCTGAGTATGTGGCTCTATGTTGAACCT
 TTGAAAGCTTGCTCTTAACCTGCTTGTGTTGGCTTCTGTAAACCTGCTGTGATAAAGTAATAAAGAGGAGGAGTCAATTTGCCCTAA
 CGGACCCGCTAGATGAGTGGGTGATCCAGAAAATGTTGAACACATATCTTTGTGGAACATATCTTCCCTCCACCCCGCAAC
 ATGGCCAAATGTGTAACTCTTGAANCAAAATTTAAATAATTTGGTCCACGAAGCCGGGCTCTCGAAGTTTAAATGACTGG
 TTGTGTGATTTTGAATGATTTGTTTGAATAGCGCGGCTTGTGTGAACCCCATAAAAGCTGTCCGCACTGCACACT
 CGGGGGCGCGAGTCTCTACCCCTGCGGTGTGTACAGCTGTGGGCCACAGCGGCTTGGAAATAAACTCTCTTGCTGTTT
 GCATCAAGACCCGCTCTGTTGAGTGATTAAGGGGAGTGGCTTTCCGAGGCTGGAGGTTCTTTTGCTGATGTTTCAATT
 TGGGGCTGTGTGGGATGCTGTCGGCCACCTTAAACCCGGAGAGCGACTTTGGAGTTAAAGGATGCTCTTTTAA
 COTGTATGATATGATACCGCGCGGCTCTGTTCTGAGTGTGTTTTCAGTGGTGGCGGCTTTGCTGTTTTCAGCTGTCTCT
 CTCAGACGTTAAGGACTGGGGGACTGTGATCAGCAGAGCTGTCCAGGAGTACACAGGCTGCCACTCTGGGGGAGCGCCCG
 GAGGTTGGGAGAGGACGAGCGCGCTGGTGGTCTCTCTGTGTGCTGAGGAGGACGAGTCTGTTGTTGTAAGCGAAAGTCT
 TCCCTCTCCCGCTGCTGCTGACCTCTTTGCTGTCTGTGGAAGACGGGAGCGGTCGGTGTCTGTGATATCTGTTGTTT
 GTTTTGTGTGTCTTCTGCTGCTCTTGTCTACGCTTTAATAAGCAGAGCTGTGAGAGCTGTGAGCTTATGTTG
 ACTCTGCACCATTTGACTGAAGTTAAATCCAGGCTCTAATTTGTTCAGTTACAGTTAAGAGGAGCACTTGCAGCACTT
 CTGTGTTCTGTAATGGCGCATCTCGATGTGGATGGCCATCAGAGGGGACCTTTAATCTGAGATATCTCGTGGCTGTA
 AAGCAATATTTTTCAGACTTGACCGGCTCTCTATCCCAATAGGAGCCCTATATCTGCTGAGAGTCTCTATAATGGAG
 GATCTCTCCGCTGCTGCTGCTGCTTCTGCTGTCTGTGGAAGACGGGAGCGGTCGGTGTCTGTGATATCTGTTGTT
 CAACACTCCGGCTGAAACAAAGTCAAGCCCTCTCTCATATCTACCCGAGATTAAGAGGCGCCGCTTGGCGGAGAACCC
 AATCTGTTCCGCAACCTTTATCTGGCACAGGGTGTCTGGAGGGGACCTCTGCGCTCTGGAGCTTGGCGGTGGAG
 GGACCTGTCTCGAGGAGCTCGAGCCCGAGGGGCGCACCCCGAGCGGACAGAGAGATTCGCGACATTACGCTGCCGAC
 GTAGCGCCCTCCACACCGCGGGGCCAATTCGAGCCCTCGATGATGCGCTTGTGGGATGCTGCTATATGTAATGGA
 AAKTAACTATCTAGGCTGAGGCTGACGCCCTTTGATCCCACTCAGAGGCCAAAGAGCTCGAGCTTGGCTGTTTATG
 ACTGGGATGATGTTGCAACAGCTCTCTCAGACACTCTTCAACCAACGAGGAGCGAGAGAGATTTCTGTAGAGGCTGAGAA
 AAATGTTTCTGGGGCGAGCGGCGACCCAGCAGTTGCAAAATGAGATTGACATGGGATTTCCCTGTACCTGCCCGGTT
 GGGACTCAACACCGGCTGAAGGTAGGGAAGCTTTGAAATAATCTATCCCAAGCTCTGTTGGGGGCTCTCGGGGCGGCTCA
 AGAGCGCCCACTAATTTGGCTAAGGTAAAGATGATGATCAGGAGCCGAAATGAACCCCTCTGTTTCTTTGTAAGGCT
 CTGGAGGCTCTAGGCTGAGGCTGAGCCCTTTGATCCCACTCAGAGGCCAAAGAGCTCGAGCTTGGCTGTTTATG
 GACGCTCAGCTCTGGATATTAGAAGAAGCTTTCAGAGACTGGAAGGGTTACAGGAGGCTGATGATACGTTATAGTGAAG
 GAGGCGAGGAAAGTATTATGAAGAGGAGACAGAGAGAAAGGGAACAAAGAAAGAGAGAGAAAGAGAGAGAGAGGAA
 GGAAAGACGATATAAAGCGGCAAGAGAGAATTTGACTAAGATCTTGGCTGCAGTTGTTGAAGGGAAGAGCAATACGGAA
 GAGAGAGAGATTGAGGAAATAGGCTAGGCGCTGACAGAGTCAAGGAGCCCTGAGACCTGAGGCTGCTGCTGTTTATG
 CAATGTGATATTGTAAGAAAAAGGACACTGGGCAAGGAACCTGCCCAAGAGGGAACAAAGAGCTAGAGGCTTTATGC
 TCGAAGAGAGATAAAGCAAGGAGAGCGGGTTCGGAGCCCTCCCGAGGCCAGGATTAAGCTTGAAGGTGGAGGGGC
 ACCAATGTAGTGTGTTGATACCGGAGGGAACATTCAGTCTACTACGCCCATTAGGAAACATAAAGATAAAAAA

TCCTGGGTGATGGGTGCCACGGGCAACACAGTATCCATGGACTACCCGAAGAACAGTTGACTTGGGAGTGGGACGGGT
 AACCCACTGCTTGCTGTACATCTCGAGTGCCAGCACCCCTCTTAGTGGAGACTATTGACCAAGAATGGGAGCACAA
 TTCTTTTGAACAAGGGAACACGAAGTGCTGCAAAATAACAACCTTCACTGTTGGTGGCCCTCCAACTAGATGACGAA
 TATCGACTATACCTTCCCTAGTAAAGGCTGATCAAAATATACAATTCTGGTGGACAGATTTCCCCAAAGCTGGGACGA
 AACCGACGATGGTGGTGGTGAAGCAATTCGCCCAACAGTTTCAATGTAAGGGCTGCGCCACAGGCTTCAGTCACTGA
 GACATACCTCTTGATGAAGAAGCTCAAGAAAGGAAATGGCGGCAATGCCAAGAATTAATCCAAAGCGGATCTTGCT
 CTCGTGCAATCTCCCTGGAAATCTCCCTGTGTACCGGTTAGAAAGGCTGGGACTAATGACTTGGCCAGTACAGGACCT
 GAGAGAGGCTCAATAAAGGCTGACGATATACACCCACAAGCTCCCGAACCTTATAACCTCTTGTTGGCTCTGCCACCC
 AACGGAGCTGGTATACAGTATTGGACTTAAAGGATGCTCTTTTCTGCTGAGATTACACCCCACTAGGCCAACCACTTTT
 CCTCTGAAATGGTATGATGACAGTACAGCTCTGGGCTGACCGGCTCCCGCAAGGCTTCAAGGAATCTTACCCATAACC
 CCGGACACTTTTGACAAAGGCTCTACACAGAGACCTGGCCAACTGACAGGATCCCAAGAGCTTGAGGACGCTCTCCATG
 ACCTGGTAGCTACCTGCTTGGCGGAGGCCACCAACAGGACTGCTTGAAGAGCCAGAGGCACTACTCGTGGATTTGTCT
 GACCTAGGCTACAGAGGCTCTGCTAAGAAGGCCAGTATGGTGCAGGAGAGGTAACTACTTGGGTCACAGTTTGGGGGA
 CGGGCAGCGATGGCTGACGGAGGCCAGGAAAGAACTGTAGTGCAGATACCGGCCCAACCCAGCCAAACAAAGTGAAG
 AGTTTGTGGGACAGCTGGATTTTCCAGACTCTGGATCCGGGCTTTCCGACCTTACGACCCCACTTACCCACTAAC
 AAGAAAGAAAGGGGAAATCTCTCTGGGCTCTGAGCAACAGAAAGGCAATTGATGCTATCAAAAGAGGCCCTCTGAGGCC
 TCTCTGGCCCTCCCTGACGTAACTAAACCTCTTACCTTTATGTGGATGAGCGTAAAGGAGTAGGCCGGGGAGTTTAA
 CCCAACTCTAGGACCAATGGAGGAGACCTGTTGCCCTACCTGTCAAAAGAAAGCTGATCTGTTAGCCAGTGGTTGGCCGTA
 TGCTTGAAGGCTATCGACAGCTGTGGCCATCTGTGTAAGGACGCTGACAAATAGACTTTGGGACAGAAATATAACTGTAT
 AGCCCCCACTGGTGTGGAGAACTGTTGGGACGCCCTCAGACGATGATGATACCAAGCCCGCATGACGACTATGCAAA
 GCTCTGCTTCTCAGAGAGGCTACGTTCTGCTCCACAGCCGCTCTCAACCTCGCACTTCTGCTTGGCTGAAGAGACTAT
 GACCAAGTGACTCATGATTGCCATCAACTATTGATTGAGGAGACTGGGGTCCGCAAGGACCTACAGACAATACCGCTGAC
 TGGAGAAAGTGTAACTGGGTCCTACGACGGAAGCAGCTATGTGGTGGAAAGTAAAGGATGGCTGGGGCGGGCTGGTG
 ACCGGGACCCGACGATCTGGGCCAGGAGCTCGCGGAGGAAGCACTTACGACCAAAAGGCTGAGCTCATGCCCCCTACGCA
 GCTTTCGGGCTGCTGACGAGGCTGACGAGTATAAAATTTATGATGATAGTATGCTTGGCACTGCGACAGCTACAGG
 GGGATCTATAAGCAAGAGGCGCTGCTTACCTCAGCAGGGAGGGAATAAAGCAACAAGAGGAATTTAAAGCTATGAT
 AAGCCTTCATTTGCCAAAGAAAGCTAGCTATTATACACTGTCTGGACATCAGAAAGCCAAAGATCCCATTTCCAGAGG
 AACAGATGGCTGACCGGGTTGCCAAGCAGCGAGCCCGAGGTTTAACTCTTGGCTTATGATGAAGAACCCCAAGCGCC
 AACCCCGACCAAGCACTACCCCTGAGAGCTCGCCAGAGATAAAGAAAGATAGACCACTTCTTGAGACTCCGGAAGGGA
 CTCCTGATACGATGAGAAATCTCTGCGGCTGAGGATGAGGATGAGATACACATCATGATGAGTGAAGTGAAGTGA
 CACCTTAGGAATAAACCTCGCAGCACTGGTTCAGCAACTCTCTATCATGTTCTGAGGCTACCGAGGATGCTGATTC
 GTGGTGCAAACTGTGTGCCCGGACGCTGGTTAATGCTAACTCTCCAGAACTACCTCCAGGAAGAGGAAATAGGGGAA
 GCCACCGAGCCGCTCTGCGGAAGTGGACTTCACTGAGTAAAGCGGGCTAAATACCGGATGAAATATCTATGTTGTT
 GTAGACACTTTTGAAGTGGTGAAGGACTTCTCTACTAAGAAAGAGACTTCAACCGTGTGGTCAATAAAGATACGTA
 GGAATTTCTGAAGAATTTGTAATACCTAAAGTAAATAGGGTCCAGCAATGTTCCAGTCTTCTGTTCAAGTGGG
 GACTGGCCCAAGATATGGGGATTTGATGGAACTGCTATTGTGCTACAGACCCCAAGCTCAGGACAGTACCGAGTATG
 AATTAAGACCAATAAAGAGACCTTACTAAATGACCGCGGAGACTGGGTTAATGATTGGATAAGCTCTCTCGCTTGT
 ACTCTTTAGGGTTAGGAACAACCCCTGGACAGTTTGGGCTGAGCCCTTGAATTTACTACGCGGGGACCCCACTATGG
 TAGAAATTTGCTTCGTGATAATGCTGACGCTGCTGCTTCCAGCTTTGTGTTCTAGGCTCAAGGCACTTGAAGTGGTG
 AGACAAAGAGGCTGGAGCACTTCGGGAGGCTCTACAGGAGGAGACTTCAGATCCCACTGCTGTTCCGCGAGTAA
 AGATTCAGTCTACGTTAGACGCCACGCTGACGGAACCTCGAGACTCGGTGGAAGGGCCCTTACAGTATTGTGACCA
 CACCAACCGCTTGGAAGTGCAGAGGAATCTCCACCTGGATCCATGCTCCACGTTAAGCCGGGCCCACTCCCGATTCG
 GGGTGAAGAGCTCTCAGCAAGACGAGTAAAGTCTCCCTTAAGCTTGGCTGCTCATCGCTGGTGTCTTACTCTGTCAATACTCTC
 AAGTATAATGTTAAGCACTGTGACAGCCGGAATCCCAATAACACTTACTCTGACCTGGTATCTACTGACTCTCGGT
 ACAGTATTAATAATTAACAGCACTCAAGGGGAGGCTCCTTGGGAGACTGGTGGCTGATTAATATCTCTCTGTGAT
 AGTAACTCCCTGGTCTCAATGACAGGCCACCCGCGATGACTCGTGCTACGTTACGGGTTTACTGTTTCCCGAGGCC
 CAAATAATGAAGAATATTGTGGAAATCTCCAGGATTTCTTTGCAAGCAATGGAGCTGGTAACTCTTAATGATGGAAT
 TGGAAATGGCAAGTCTCAGCAAGACAGAGTAGTATTCTTTTGTGTAACAACTACCAAGTATAACCTAATTAATTA
 TGGCAATGGGAGCACTGATGATGATCTGTAAGGCTGGAGGGCAACGATGATGATGATGATGATGATGATGATGATGAT
 ACCTAGATTCTTAAATAAGTTTCACTGAAAGAGGAAGCAAGAAATAATCAAGCTGATGATGATGATGATGATGATGAT
 GGAATAGTGTACTATTGGAGGCTCTGGGAGAAAGAAAGGATCTGTTCTGACTATTGGCTCAGATAGAAATCAATGATGA
 ACCTCCGTTGCTATATGAGGACCAATAAGGTTTGGCGCAAGAGGACCTCAATCCAGGAACAGAGGCCACTCTTAAC
 CCTCTGATTACAACTACAACTCTGATGACTTCCCACTGAGCTTAACATCACTATAAAGAGGGGCGAACTTTTCAAT
 CTATCCAGGGGAGCTTTTCAAGCTCTTAACCTCGAGCTCGAGGCTGAGCTCTCTCTGTTGGCTTGTGCTTCACTGGG
 CCCACCTTACTATGAGGGAATGGCTAGAGGAGGGGAATTCATGTGACAAAGGAACATAGAGCAATGTACATGGGAT
 CC

LamA10

GATCTGGGTGCAGTGGTGTGAAGGGAAGCAATACGGAAGAGAGAGAGATTTAGGGAATAATAGGTCAAGCCCTAGAC
 AGTCAAGGAACCTGGGCAATAGGACCCCACTGACAAAGGACCAATGTGCATATTGTAAGAAAGAACGACACTGGGCAAG
 AACTCGCCCAAGAAAGGGAACAAAGCTGAGGTTCTAGCTCTGGAAGGAAGATAAAGACTAGGGAAGCAGCGGCTTCGGA
 CCCCCTCCCGCTGAGCACTGAT
 CAGTGTCTACAGGCAATAGGAAGAACTAAAGAAATAAAAACTTCCGAGTCTGGGTGAAGGCTGAGTGAAGCAAGCACTGCA
 TGACTACCCGAAGAACAGTGTACTTGGGAGTGGGAGCGGTAACCCACTGTTTGTGTCATACCTGAGTGCCCAAGCC
 CCTCTTAGTGAAGACTATTGACCAAGATGGGAGCACAAAATTTCTTTGAAACAAGGGAACAGAAAGTGTCTGCAAT
 ACAACCTATCAGCTGTTGACGTCACAAATGATGACGAATGACTTACTTCCCTAGTAAGAAGCTGATCAAAAT
 ATACAAATTCCTGTAAGCAAGTTTCCCAAGCCCTGTAAGCTGATGATGATGATGATGATGATGATGATGATGATGATGAT
 AGTTATTCACTGAAGGCACTGTCACCACTAGTTCAGTACAGCACTACCCCTTAGTATGAAGAACTGAAGGAATTC
 GCGCGCATCGCAAGATAATCAACCAAGGGGACTCTAGTTCTGTCCAATCTCTCGGTAATCTCCCTCGTCACTCGGTT
 AGAAAGGCTGGCACTAATGACTATCGACCACTGACAGGACTTGAGAGAGGTCAATAAAGCGGTGAGGATATACCAACAC

LATA11

[illegible]

Lama3A

[illegible]

ACCTTGGCGAAGCTTTCTGTGTCTCTGAATGGCCGACATTCGAGTGTGGATGGCCATCAGAGGGGACCTTTAATCTGGAGA
TTATCTGGGCTGTTAAAGCAATATTTTTCAGACTGGACCGGCTCTCATCCCAATCAGAGGCCCTATATCTTACGTGGT
CAAGATTTGGTCAGGAGGATCTCCGCCATGGGTTAAACCTTGGCTGAATAAGCAAGAAGAGGCCCTCCGCAATCTCCGG
TCTTGGAGAGAAAAACAACACTCGGCTGAAAAAAGTCAAGCCCTCTCTCATATCTACCCCGAGATTAGAGGAGCCGGCG
CTTGGCGGGAACCCCAATCTTTCGCCACCCTCTTATCTGGCAAGGGTGTCCGAGGGGACCTCTTGGCCCTGTGGAG
GCTCCGGCGGTGGAGAGAGCTCTGGAGGAGCTTGGAGGCTCGGAGGGGCTCCACCCGAGCGGAGAGAGAGAGAGAGAG
ATGACCTCTGCCACCTGAGCGCCCTCCACACCGGGGGGCCAATTGCGAGCCCTCCAGATTGGCCCTTTCTTCTCGAG
ATCTCTAATAATGGAAAACTAACCACTCCCTCTTCTCGGAGGATCCCAACGCTCCAGGGTGTGGAGTCCCTATTGT
TTCTCTTCCAGAGACTCTTGGGATGTTGTCAACAGCTGCTCCAGAGCACTTCTTCAACCAAGGAGGAGCGAGAGAGAAATCTT
TTAGAGGCTAGAAAAATTTCTTGGGGCCGAGCGGCCACCCAGCTTGGCAAAATGAGATGACATGGGATTTCTCT
TGACTTCCGCCCGGTGGCATACACAGCGCTCAAGGTTAGGAGAGCTTGAATAATCTTCCGACGCTCTGTGTGGCGGT
CTCCGGCGGCGCTCAAGACGGCCCACTAATTTGGCTAAGGTAAAGGAGAGTGTGAGGAGGCCAATGAAGCCCTCTGT
TTTCTTGGAGAGGCTTGGAGAGCCTTACGGGCTACACCCCTTTTGTATCCACCTCAGAGGCCCAAAAGGCCCTCAGTG
CTTGGCTCTTATAGGACAGCTAGCCTTGGATATTAGAAAAAGGCTTCAGAGACTGGAAGGTTTACAGAGGCTTGAGTTA
GTGATCTAGTGAAGAGGAGGAGAAAGTATATTACAAAAGGAGAGAGAGAGAAAGGAGCAAGAAAGAGAGAGAGAG
AAGAGAGGAAAGGAG
AAGCACTACGGAAGAGAGAGAGAGATTTAGGAAAAATAGGTACGGCCCTAGACAGCTAGGGAACCTGGGCAATAGGACG
CCACTGCACAGGACCAATGTGCAATTATTAAGAAAAAAGGACACTGGGCAAGGAATGCCCAAGAAAGGGAACCAAGG
ACTGAAGGTTTGTAGCTCTGGAAGAGATTAAGAGACTAGGGAAGAGCGGGGTTCCGAGCCCTCCCGAGGCCCAAGGTAATCT
TGAAGGTTGGGGGCAACAGTTGGGTTCTGGTTGTATGACCCGAGCAAAACATTCAGTGCTACTACAGGCTATTAGAAAA
CTAAAGATTAATAAAATTTCTGGGTGATGGGTGCCACAGGCAACACAGTATCCATGGACTACCGGAAGACAGTTGACT
GGGATTTGGGACGGTTACCCCACTGGTTCTTGTGTCACTACTGAGTGCCACAGCCCTCTTATGAGTAGAGACTTATGACCA
AGATGGGAGCACAATTTCTTGTGAACAGGGAACAGAGAAAGTGTGTGCAATAACAACTACTACTGTGTGGACCTC
CAATATGATGAGCAATATGCACTATCTCTCCCTGATGAAGGCTGTGCAATAATACATTTCTGTGTGGAGCAAGTTCT
CCAGGCTGAGGAGCAAGAACCGAGGATGGGTTTGGCAAGCAATCTCTCCCAACAAATATTACAACTGAGGAGAGAGAG
CACTGTGTGCTGACCACTGAGTGAATGAAGAGCTTGGGAGAGATTTGGGAGAGATTTGGGAGAGATTTGGGAGAGAT
CAGGGCTACTAGTTCTTGTCCAATCTCCGTGAATATCTCCCTGCTACCGGTTAGAAAGGCTGGGACTAATGACTATG
ACCACTACAGAGCTTAGAGAGGTTCAATAAAGCGGTGCAAGGATATACACCACTCCGACCTTCTTAACTCTTGT
GTGCTCTCCCAACCAAGCGGACTGGTATACAGATTAATGAGTATTAAGGATGCTCTTTTCTGCTTGAGATACACACCACT
AGCAACCACTTTTGGCTCTGGAATGGAGAGATGATTTGGAGAGAAAGGCGCACTCTGGAGCCAGCTGAGCAAGCTT
AGGTTCTAAGAGGAG
TGACCTCTCCCAAGTACGTTGAGTGACCTGCTTCTGGCGGGAGGCCCAACAGGACTGTTTGAAGAGGACAGAGGCACTA
CTGCTGAATTTGTGATCACTAGCTACAGAGCTCTGCTAAGAAAGGCCAGATTTGAGAGAGAGAGGATTAATATCTGG
GTACAGTTTTCGGGAGCGGCGAGCGATGGCTGACGGAGGCCAGGAGAGAAATCTGTAGTCCAGATACCGGCCCCAACCCAGC
CCAAACAAATGAGAGATTTTGGGAGCAGCTGGATTTTGGACAGCTTGGATCCCGGGGTTTGGAGCTTAGCAGCCCA
CTCTACCCACTACAGAAAGTAAAGGAGATTTCTGTGGCTCTGAGAGGAGAGGCAATTTGATGCTTCAAAAGGCG
CTCTGAGGAGCACTGCTCTGGCCCTCCCTGACGTAATAACCCCTTACCTTTATTGTGGATGAGCTAAGGAGGATAG
CCCGGGGAGTTTAAACCAAACTTAGGACCAATGGAGGAGACTGTGGCTACTTCAAGAGAGCTCTGATCTAGGCG
AGTTGTGGCCCGTATGCTCTGAGGCTATCGGACGCTTGGCCATCTGGTCAAGGAGCGGTGACAAATTTGACTTTGGGACA
GAATATAACTGTAAAGCTCTCTCTCACAGAGAGGGTCACTGTGGCTCCACCGGCTCTCAACCTGGCACTCTTCTG
CTTGAAGAGAGACTGAAGAACAGTGACTATGATGCTCACTAATTTGATGAGGAGACTGGGTTCCGCAAGGACCTTAC
AGACATAACCGTGAAGGAGAGAGGATTTAACTGGTTCTACTGAGGAGAGAGCTATTGTGGTGAAGTTAAGAGGATGGCTG
GGGCGCGGTGGTGGAGCGGAGCCGACGATCTTGGGCGAGCAGCGCTGGGAGAGACTTACAGCAAAAGGCTGAGCTCT
ATGGCTCTACAGCTTCTGGGCTGCCAGAGGAAATCCATAAGAGCACTTATACAGAGAGAGAGAGAGAGAGAGAGAGAG
TGACCACTGACAGGCGGCACTATAAGCAAAAGGGGTTGCTTACTCTACGAGGAGGAGAAATAAGAAACCAAGAGAGAA
TCTAAGGCTATTAGAAGGCTTACATTTGCCAAAAAGGCTAGCTATTATACACTGTCTCGGACATCAGAAAGGCAAGAT
CCCATATGCCAGAGAACCAAGATGGCTGACCGGTTGCCAAGCAGGCGAGCGGAGGTTAACTCTTGCTCTATTAGATA
AATCAACAAAGGCCAGAACCCCGGAGCAGATACACCTTAGAGAGCTGGCAAGATATAAAGAAATGAGACCAATCTCTGT
AGACTGGGAGAGGAGCTCTATCTCTAGAAGAGAGAAATCTTCCCAACCTTCAAGGTTAGAGTATGAGTCAAGGAG
TACATGTTCTCAAGGCTACTAGAACCAATAAACACCTGACAGCTTGTGTGACAGACTCTCTTATATGTTTGTAGGACTC
AGGATGGGCTAGTTGGGTGGTCAACCACTGTGTGCCCTGCCAGCTGTTAATGCTAATCTCTCAGAAATACTCTCAGGAA
AGAGACTAAGGAGAGGAGCCACCGGCTCTACTGGGAAGTGGAGTCTACTGAGTAAAGCCGCTTAAATCAGGAAACAA
TATCTATTGGTTTGTGAGACCTTTTCAGGATGGTGAAGGCTTATCTCTAAGAAAGAGACTTCAACCTGTGGTGGC
TAGAATAATCTGAGGAAATTTTGTGACAGATTTGTGACAGATTAAGGTTAGATATAGGCCCGGCTGTGTT
CCAGGATGAGCTCAGGAGCTGCGGCAATATTTGGGATGATTTGGAAATCTGATAGGCTGAGCAATGGTGGCTGAGT
CAGGATGAGAGGATGATTAAGAACCTTAAAGAGCCCTTACTAAATGACCGCGGAGAGCTGGGCTTAATGATGGGATAGC
TCTCTGGCTTTGTGCTTTATAGGTTAGGAACACCCCTGGACAGTTTGGGCTTACCCCTATGAATTTACTCTAGGAG
GACCCCGCCCAATGGTAGAAATGACTGTGATGATGCTGAGGCTGCTTTCCCAAGCTTTGTCTCTGAGGCTGAG
GCATCTGAGTGGGTAGAACAGAGGCTGAGAGCACTCCGGAGGCTTACTCAGGAGGAGAGACTTTCAGATTC

LamA6

TGGAATTGCGAGCTGTGAGTCCCGGGTTTGGGACCTTAGCAGGCCCACTCAACCACTAACCAAGAAAAAGGGGAAT
TCTCTGGGCTCTCGACACAGAGGCAATTTGATGCTATCAAAAAGGCCCTGCTGAGGCACTGCTCTGGCCCTCCCT
GAGTCACTAAACCCCTTATCCCTTATTTGGGATGAGCGTAAAGGAGAACCCCGGGAGATTATTAACCAAGCTACTAGGAC
ATGAGAGAGGACCTTGTGCTACCTGTGCAAAAGAGCTGATCTCTGAGCGAGTGGTGGCGGCTATGCTCTGAGAGGATCTC
CAGCTGTGGGCTATCTGTGCAAGAGCCCTGACAAATGACTTTGGGACAGATATTAAGTGAATATAGGCCCGGCTGTGTT
GAGACATCTCTGAGCCCTGAGCGCTGATGATGACCAAGAGGCTGAGCCACTTCAAGCTCTTCTGATGCTTCTGATG
GAGGCTACAGTTTGTCTCACCAGCGCCCTTCAACCCCTGCCACTCTTCTGCTGAAGAGACTGATGAACAGCTGACTCATG
ATTGCCATCACTATTTGTGAGGAGCTGGGCTCGCAAGGACCTTACAGCATACCGGTATGGAGATGAGGATTTAAAC

TGTTTCACTGACGGAAAGCACTATTTGGTGGAAAGTAAAGGATGGCTGGGGCGGGCGTGGTGGACGGGACCCGACGAGT
 CTGGGGCCAGCGCGCTGGGGAAGGAAGCTTCAGACCAAAAGCGTGAAGCTCTAGGGCCCTGACGGAAGCTTTGGCGGGC
 AAGGGAAATCACTAAACATTTATACAGACAGCAGGTATCGCTTTGGGACTGCACACGTACACGGGGCCATCTATAAGCAA
 AGGGGGTTGCTTACCTCAGCAGGGGAGGGAAATAAAGAACCAAGAGGAAATCTAAAGGCTATAGAAGCCTTACATTTGGC
 AAAAAGGTCAGCTATTATACACTGTCTCTGGACATCGAAGAAAGCAAGATCCCATATCCAGAGGAAACACAGATGGCGTACCG
 GGGTGGCAAGCAGCGCAGCCAGAGGGTTTAACTCTTCTGCTATGATAGAAGACATCCCAAAAGCCAGCAACCCGAGCAGCAG
 TAGACCTGAGAGCTGCGAAGGATAAAGAGTAGAGAGTTCTCTGAGACTCTCGGAAGAGGACTCTGTATACCTTCAGCA
 TGGGAAGGAAATCTCGCGCCACAAGAAGGGTTAGAATTATGTCCAAACGATACATCGTTCAACCCACCTACGAAGCAATAAC
 ACCTCGACAGCATGTGTGCAGACATCTCCTTATCATAGTTCTTGAGGCTACCAAGGATGGCTGATTCGGTGTGCAAAACACTGT
 GTGGCTTCGGCAGTGGTTAAATGTATATCTCTCCAGAAATACCTTCGAGGAAAGAGCATAAAGGGAAGCCACCCAGCGGCTCA
 CTGGGAAGCTGGACCTTCACTGAGGTAAAGCGGCTATAATACCGGACCAATAATCTATGGTTTATTGTAGACAGCTTTGAC
 GATGGGTAGAAGCTTATCTCTATAAGAAAGAGACTTCAACCGTGGTGGCTAAGAAATAATCTCGGAGGAAATTTTTCGACGA
 TTTGGAATCACTAAGTATAAGGTGCAGACAAATGGTCCAGCTTTGCTTGGCCACGGTAAGTCAGGGACATGGCCAAAGATT
 GGGGATGTATTGGAAAGATCGATATGTGCAATACAGACCCCAAGAGCTCAGGACAGGTAGAGAGGATGATATAGATTTCAAAATGAAAG
 AGACCTCTACTAAATTAAGCCCGGAGACTGGCGTTAATGATTTGATAGCTCTCTCGCTTTAGGTTTATGGGTTAGG
 AACACCCCTGCAGAGTTTGGCTGACCCGCTATGATATCTACCGGGGACCCCGCCATTTGGTGAATAATCTTCCTG
 ACATAGTGTGACGTGGCTCTTCCAGCGCTTTGTGCTTAGGCTCAAGGACATCTAGTGGGTGAGACAGAGCGCTGGA
 GGCAACTCGGGAGGCGCTCACTAGGAGGAGGAGACTTGCAGATCCCAACATCGTTTCCAAAGTGGGATTCAGTCTACCTG
 AGACCGCAACCCGTGACGAAACCTCGAGACTCGGTGGAAAGGGCCCTATACAGCTACTTTTGACCAACCAACCGGTGTGAA
 AGTCAAGGAATCTCCAGCTGGATCATGCTATCCCATTAAGCCGGCGCCACCTCCCGATCGGGGTGGGAAGCCGGA
 AGACTAAATAATCTTAAGCTTGGCTCCATCGCGTGGTCTTACTCTGTGCAATAACTCTCAAGTTAATGGTTAAAGC
 CCTTGTGGACAGCCGGAATCCCAATAAACCTTATCTCTCACTGGTTACTTACTGACTCCGGTACAGTTAATAATATT
 ACAGCACTCAAGGGGAGCTCCCTTGGGGAACCTGGTGGCTGAAATATATGCTGCCCTTGATGATCAATGCTGGCTGCTC
 AATGACAGGCCACACCCCGGATATCTTCAAGCTGCTTACGGGTATCTCCGCTTGGCCAGAGCCCGCAATAATGAGAATA
 TTGTGGAAATCTCAGGATTTCTTTTGAACCAATGGAGCTCGGTAACCTTCTAATGATGGGAAATGGAAATGGCCAGTCT
 CTATGAGGAGATCTTCTTGTATGATTTCTTGTAACTATCTCAAGGATATGATCAATTAATTTGGGAGGAGGAGGAGG
 AAGATGTGGCAAGCGGGTACAAAAAGATGTACGAAATAAGCAATAAGCTGTCAATTCGTTAGGACTAGATTAATTA
 AATAAGTTCTACTGAAAAAAGGAAAAACAAGAAAAATTCAAAAGTGGGTAATGTGATCTTTGGGCAATGTGTACTATG
 GAGGCTCTTGGGAGAAAGAAAGGATCTGTTCTGACTATTCGGCTCAGAAATAGAAACCTGACATGGAAAGCTTCGGTGTGCTATA
 GGACCAATAAGGTTTGGGCAACAGAGAGCTCCCAATCCAGAACAGAGGCACTCTCTAAACCCCTGATTTAGCAATAC
 AACCTCTGGATAGGCTCCAGCTGAGCTAAATCACTATTAAACAGGGGCGAACTCTTTTAATCTCTGACAGGAGCTT
 TCAAGACTTCTAACTCCAGCACTCAGAGGGCTACCTCTCTGTGTGGCTTGTCTAGCTTTGGGGCCCACTTACTAATG
 GGAATGGCTAGAGGAGGGAATAATCAATGTGACAAAGGAACATAGAGACCAATGTACATGGGAGTCCCAAAATAGAGCTTAC
 CCTTACTGAGGTTTCTGGAAAGAGCCATGCATAGGGAATGGTTCCGCCATGCCACCAACCTTTGTAACCACTACCTGAAG
 CTTTAAATCCAACTCTGGAGTCACTATCTGTGATCTGTTATGACAGGTGGGCTAGTGAATCTGTGTAGAGCT
 TGTGTTTGCACCTTTGTAACCAACTAAGACTTTTGGGTATTTGGTCCAAATTTGCCCGGTTGTACTATAATCC
 CGAAAAAGCAGCTCTTGTAATATGACTATAGATATAATCGGCCAAAAAGAGAGGCCATATCTCTGACACTAGCTGTTA
 TGCTCGTATTTGGGAGTGGCTCGAGCGTGGGAACAGGAACCGCTGCCATAATCAACAGGACCGCAACCTGGAAGAAAGGA
 CTAGTAACTACATCGAATTTGTAACGGGAAGATCTCCAAGGCTAGAAAAAATCTGTAGTAAGCTTGGAGGAAATCCCTAAC
 CTCTTATCTGAAGTGTGTACAGAGCAAGAGGGGTATGATCTGTTATTTCAAGAGAGAGGAGGATTTGTGTAGAGCT
 TGAAGGAGGAATGTGTGTTTATGAGATCTATTCCGGGGCTACAGAGACTCTGAACAGCTTGAAGAGAGCTTGTGAG
 AAGCTGTCAAGAGGAAAAAGGAACTCACTAAGGGTGGTTTGAAGGATGGTTTCAACAGGTCTCTCTTGTGTGGCTACCTACT
 TCTGCTTATCAAGAGCCCTAATAGTCTCTCTCTGTTACTCAGAGTGGGCGATGTTATTAATAACAGATTAATGTGCT
 TCAATAGAGACGAAATAGTGCAGTCCAGATCATGTGACTTAGACAACAGTCCAAAGCCGCTTAGCAGAGAAAGGTGGC
 CCTAGCTCTACAGGTTCTAGATGATGAACATTAACAGAGGAAGATGGGAATGCAAGAGGATGAATAATGCAACTGTG
 TCTCCAGAACACAGGAAGTAAATAAGAGCTCTAAATGCCCTCGAATCTAGCCAGCCCTTCTGCTATAGGTAAGTAC
 TACTTTTGTGCTTTTAGGGCTTGTCTTCTGCTCTGTACAAAATTTTGTGGAAGAGGAAAAACAGCCCTCGATATGTG
 CCTTATGTCTTGAACCTCTTGAACCTGCTCTAAGCTGCTGTTTGGGCTCTGAAACCTGCTGCAATAGATAAGAAAAAG
 GAGAAGTCAATTGCCATAAGCGACCCCAAGTAAAGATCGGGTGTACCAAAATAATGTTGAACACATATCTTTGTGACACAT
 GTCTCCGACAGCCGGAAGCAATGTGATGATATGTAACAGAGCTCTGCAACATTAATTAATTAATTAATTAATTAATTAAT
 GAAGTTTAAATGTGACTGTTTGTGATATTTTGAATAATGTGTTTAAAGCGGGCGCTTTGTGTGAACCCCAATAAA
 CGTGTCCGACGCTCCACCTCGGGCGCAGTCTCTACCCCTGCGTGTGTACGACTGTGGGCGCAAGCGCTCTGGAAT
 AAAAATCTCTCTGTGTGATCATAGACCGCTCTCTGTGATGATTAAGGGGAGTCGCTCTTTTCGAGCTTGAAGGTT
 TTTTGTGACTGTACAGGACCTTTATTTTTCCTATT

LamA8

GGATCTGTGGTTTCTGTTTGTGRTGCTTTGTCTGTGGCTCTTGTCTACAGTTTAAATAGGACAGAGCGGTGACGA
 CGCCTCTTGTGTGCTGTGCAATTTGGAGCTGACCTTAAATCCAGGCTCAATTTGTCTAGTTTCAGGTTTAAAGAGGGGA
 CCTTGGCGAGCTTTCTGTGTCTGAAATGGCCGACATTCGATGTGGAATGGCCATCACAGGAGGAGCTTAAATGAGAT
 TATCTGGCTGTTAAAGCAATATTTTCAGACTGGACCGGCTCTCATGCCAATCAGGAGCCCTATAATCTTATCGTGGC
 AAGATTTGGCAGGAGATCTCCGCCATGGGTTAAACCTTGGCTGCAATAAGCCAGAAAGCCAGGTCCCGGAATTTGGCT
 CTCTGAGAGAAAAACAACACTCGGCTGAAAAGTCAAGCCCTCTCTCATTTATTCAGCCGAGATGTGAGAGCGCCGCGC
 TTGGCGGAACCCCAATTTGTCTCCCAACCCCTTATCTGCGACAGGAGGCTGTGCGAGGGGAACCTCTCTGCTGGA
 CTCGGCGGCTGGGGAGGACTGTGCAAGGACTCGGAGCGCGAGGGGGCCACCCCGGACAGGACAGCAGAGATCGCGACA
 TTAACCTGTGCGACAGTACCGGCTCCCAACCGGGGGGCAATTCGACCGGCTCAGATATGGCCCTTTCTTCTGCGA
 TCTCTATAATTTGGAACAAACCACTCCCTCTTCTGGAGGATCCCAAGCGCTCACGGGGTGGTGGAGTCCCTTATGT
 TCTCTGACGAGCTTGTGAGATGATTTGTGACAGCTCTGCAACACTCTTCACCAAGGAGGAGGACAGAGAGAAATCTGT
 TTAGAGCTAGAAAAAATGTTCTGGGGCGACGGGCAACCCAGTATGCAAAATGTAGATTTGACATGGGAATCTGTGAT
 GACTCGCCCGTTGGGACTCAACACGGCTGAAGTAGGAGAGCTTGAATATCTATGCCAAGCTCTGTGGTGGGGGTCT
 TCGGGCGGCTCAAGACGCCCACTAATTTGGCTAAGTAAAGAAAGATGTACGAGGAGCAATGAACCCCTCTCTTT

[illegible]

TGTTGAAGTAACCCGTGACAGGAAGCCAGCTTAGAGAAAGGTTGGAGAAGAGCGGTGCAAGAGGAAAAGGAAATCTCTCAAGG
 TGTGTGTGGGGATGTTCAACAGGCTCTTGTTGGTGTGACCTCACTTCTGTCTTGCAAGGCGGAAATTAAGTCTCTCT
 CTTGCTTACTACCAATGTGGGGCATGTATATTTCAAGAAATTAATGGCTCTATTAGAAGAGAAATAGTGCAGACGATCAT
 TGGTATAGTACAGCAATCCAAAGAGGCTTACACGAGAAAGCTGGCCGTACCAAGCTTACCAAGTCTAAGATAGAAGATCT
 TAACAGAGAGAAAGATGGGGATGAGAGATGAAGAAAGTGAAGTACCTCTGAGAGAGAGAGAGAGAGAGAGAGAGAGAGTCT
 CAGTCTACAGCT
 CTGCAAAAACTTTGGTGAAGGGGAAALAAACAGGCTCAGATATGTCCTTATGCTTGAACCTTGAAGCAGCTCTGCTGCT
 AACTGCTCTGTTTGGCTCTGTAAGATGCTTGCAATAAGTAATAAAGAGAGAGACATTAAGTCTTACAGCGCCAGTAAG
 ATCGGTGGTACACCAAAATGTTGAACACATATCTTGTTGACCAAGTCTGCCCCAGCGGAAACATGCGCAAAATGTGT
 AACTCTAAAGATTAATTAATTAATTTGTCGACAGAGCGGGGGCTCGAAGTGTTAATATGTGCTGTTGTGATATTTTG
 AATATGATGTTTGAAGACGGGGCTTTGTGTAAAG
 TCTGCTGAGTGATTAAGGCGATGCTGCTTTCCGAGCTGAGGAGTCTTTCTGCTAGTCTTACACAGCAAGCGCT
 GTTCTAAAGAGATGCTGGCCCTCAACCCAGTCA

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **METHODS TO INHIBIT INFECTIOUS AGENT TRANSMISSION**, the specification of which:

☐ is attached hereto.

☒ was filed on November 15, 2001 as Application Serial No. 09/980,526.

☐ was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

U.S. Serial No.	Filing Date	Status
60/135,631	May 24, 1999	Abandoned

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Serial No.	Filing Date	Status

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed
WIPO	PCT/US00/14296	May 24, 2000	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

Combined Declaration and Power of Attorney

Page 2 of 2 Pages

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

(19)
Mark S. Ellinger, Ph.D., Reg. No. 34,812
Wayne E. Willenberg, Reg. No. 28,488
H. Sanders Gwin, Jr., Reg. No. 33,242
Ronald C. Lundquist, Ph.D., Reg. No. 37,875
J. Patrick Finn III, Ph.D., Reg. No. 44,109
Monica McCormick Graham, Ph.D., Reg. No. 42,600
Arlene Hornilla, Reg. No. 44,776
Greg H. Gardella, Reg. No. 46,045
Anton J. Bokal, Ph.D., Reg. No. P-51,243
John F. Hayden, Reg. No. 37,640

Dorothy P. Whelan, Reg. No. 33,814
Richard J. Anderson, Reg. No. 36,732
Stephen R. Schaefer, Reg. No. 37,927
John A. Dragseth, Reg. No. 42,497
M. Angela Parsons, Ph.D., Reg. No. 44,282
J. Richard Soderberg, Reg. No. 43,352
Chad A. Hanson, Ph.D., Reg. No. 44,737
Greg A. McAllister, Reg. No. 47,779
Ruffin B. Cordell, Reg. No. 33,487

Address all telephone calls to J. PATRICK FINN III at telephone number (612) 335-5070.

Address all correspondence to MARK S. ELLINGER at:

FISH & RICHARDSON P.C., P.A.
60 South Sixth Street
Suite 3300
Minneapolis, MN 55402

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

1-00 Full Name of Inventor: MARK J. FEDERSPIEL
Inventor's Signature: *Mark J. Federspiel*
Residence Address: Rochester, MN MN
Citizenship: United States
Post Office Address: 5722 Colonial Lane SE
Rochester, MN 55904

Date: 3/08/02

SEQUENCE LISTING

<110> Mayo Foundation for Medical Education and Research
5 Federspiel, Mark J.

<120> Methods to inhibit infectious agent transmission associated
with transplant

10<130> 150.192WO1

<150> US 60/135,631

<151> 1999-05-24

15<160> 32

<170> FastSEQ for Windows Version 4.0

<210> 1

20<211> 47

<212> DNA

<213> Artificial Sequence

<220>

25<223> A primer

<400> 1

gcgcgatgcag atctgatgct taaacaggta gaaattttca ccgatgg

47

30<210> 2

<211> 45

<212> DNA

<213> Artificial Sequence

35<220>

<223> A primer

<400> 2

gctgctgcgt cgacttaaac ttcaacttgg tagcctgtat ctctc

45

40

<210> 3

<211> 659

<212> PRT

<213> Porcine endogenous retrovirus

5<400> 3

```

Met His Pro Thr Leu Ser Arg Arg His Leu Pro Ile Arg Gly Gly Lys
 1              5              10              15
Pro Lys Arg Leu Lys Ile Pro Leu Ser Phe Ala Ser Ile Ala Trp Phe
      20              25              30
10Leu Thr Leu Ser Ile Thr Pro Gln Val Asn Gly Lys Arg Leu Val Asp
      35              40              45
Ser Pro Asn Ser His Lys Pro Leu Ser Leu Thr Trp Leu Leu Thr Asp
      50              55              60
Ser Gly Thr Gly Ile Asn Ile Asn Ser Thr Gln Gly Glu Ala Pro Leu
1565              70              75              80
Gly Thr Trp Trp Pro Glu Leu Tyr Val Cys Leu Arg Ser Val Ile Pro
      85              90              95
Gly Leu Asn Asp Gln Ala Thr Pro Pro Asp Val Leu Arg Ala Tyr Gly
      100              105              110
20Phe Tyr Val Cys Pro Gly Pro Pro Asn Asn Glu Glu Tyr Cys Gly Asn
      115              120              125
Pro Gln Asp Phe Phe Cys Lys Gln Trp Ser Cys Val Thr Ser Asn Asp
      130              135              140
Gly Asn Trp Lys Trp Pro Val Ser Gln Gln Asp Arg Val Ser Tyr Ser
25145              150              155              160
Phe Val Asn Asn Pro Thr Ser Tyr Asn Gln Phe Asn Tyr Gly His Gly
      165              170              175
Arg Trp Lys Asp Trp Gln Gln Arg Val Gln Lys Asp Val Arg Asn Lys
      180              185              190
30Gln Ile Ser Cys His Ser Leu Asp Leu Asp Tyr Leu Lys Ile Ser Phe
      195              200              205
Thr Glu Lys Gly Lys Gln Glu Asn Ile Gln Lys Trp Val Asn Gly Met
      210              215              220
Ser Trp Gly Ile Val Tyr Tyr Arg Gly Ser Gly Arg Lys Lys Gly Ser
35225              230              235              240
Val Leu Thr Ile Arg Leu Arg Ile Glu Thr Gln Met Glu Pro Pro Val
      245              250              255
Ala Ile Gly Pro Asn Lys Gly Leu Ala Glu Gln Gly Pro Pro Ile Gln
      260              265              270
40Glu Gln Arg Pro Ser Pro Asn Pro Ser Asp Tyr Asn Thr Thr Ser Gly

```


	275		280		285
Ser Val Pro Thr Glu Pro Asn Ile Thr Ile Lys Thr Gly Ala Lys Leu					
290		295		300	
Phe Asn Leu Ile Gln Gly Ala Phe Gln Ala Leu Asn Ser Thr Thr Pro					
5305	310		315		320
Glu Ala Thr Ser Ser Cys Trp Leu Cys Leu Ala Ser Gly Pro Pro Tyr					
	325		330		335
Tyr Glu Gly Met Ala Arg Gly Gly Lys Phe Asn Val Thr Lys Glu His					
	340		345		350
10Arg Asp Gln Cys Thr Trp Gly Ser Gln Asn Lys Leu Thr Leu Thr Glu					
	355		360		365
Val Ser Gly Lys Gly Thr Cys Ile Gly Met Val Pro Pro Ser His Gln					
	370		375		380
His Leu Cys Asn His Thr Glu Ala Phe Asn Arg Thr Ser Glu Ser Gln					
15385	390		395		400
Tyr Leu Val Pro Gly Tyr Asp Arg Trp Trp Ala Cys Asn Thr Gly Leu					
	405		410		415
Thr Pro Cys Val Ser Thr Leu Val Phe Asn Gln Thr Lys Asp Phe Cys					
	420		425		430
20Val Met Val Gln Ile Val Pro Arg Val Tyr Tyr Tyr Pro Glu Lys Ala					
	435		440		445
Val Leu Asp Glu Tyr Asp Tyr Arg Tyr Asn Arg Pro Lys Arg Glu Pro					
	450		455		460
Ile Ser Leu Thr Leu Ala Val Met Leu Gly Leu Gly Val Ala Ala Gly					
25465	470		475		480
Val Gly Thr Gly Thr Ala Ala Leu Ile Thr Gly Pro Gln Gln Leu Glu					
	485		490		495
Lys Gly Leu Ser Asn Leu His Arg Ile Val Thr Glu Asn Leu Gln Ala					
	500		505		510
30Leu Glu Lys Ser Val Ser Ser Asn Leu Glu Glu Ser Leu Thr Ser Leu Ser					
	515		520		525
Glu Val Val Leu Gln Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Lys					
	530		535		540
Glu Gly Gly Leu Cys Val Ala Leu Lys Glu Glu Cys Cys Phe Tyr Val					
35545	550		555		560
Asp His Ser Gly Ala Ile Arg Asp Ser Met Asn Lys Leu Arg Glu Arg					
	565		570		575
Leu Glu Lys Arg Arg Arg Glu Lys Glu Thr Thr Gln Gly Trp Phe Glu					
	580		585		590
40Gly Trp Phe Asn Arg Ser Pro Trp Leu Ala Thr Leu Leu Ser Ala Leu					

4

595 600 605
 Thr Gly Pro Leu Ile Val Leu Leu Leu Leu Thr Val Gly Pro Cys
 610 615 620
 Ile Ile Asn Lys Leu Ile Ala Phe Ile Arg Glu Arg Ile Ser Ala Val
 5625 630 635 640
 Gln Ile Met Val Leu Arg Gln Gln Tyr Gln Ser Pro Ser Ser Arg Glu
 645 650 655
 Ala Gly Arg

10
 <210> 4
 <211> 660
 <212> PRT
 <213> Porcine endogenous retrovirus

15
 <400> 4
 Met His Pro Thr Leu Ser Arg Arg His Leu Pro Ile Arg Gly Gly Lys
 1 5 10 15
 Pro Lys Arg Leu Lys Ile Pro Leu Ser Phe Ala Ser Ile Ala Trp Phe
 20 20 25 30
 Leu Thr Leu Ser Ile Thr Pro Gln Val Asn Gly Lys Arg Leu Val Asp
 35 40 45
 Ser Pro Asn Ser His Lys Pro Leu Ser Leu Thr Trp Leu Leu Thr Asp
 50 55 60
 25Ser Gly Thr Gly Ile Asn Ile Asn Ser Thr Gln Gly Glu Ala Pro Leu
 65 70 75 80
 Gly Thr Trp Trp Pro Glu Leu Tyr Val Cys Leu Arg Ser Val Ile Pro
 85 90 95
 Gly Leu Asn Asp Gln Ala Thr Pro Pro Asp Val Leu Arg Ala Tyr Gly
 30 100 105 110
 Phe Tyr Val Cys Pro Gly Pro Pro Asn Asn Glu Glu Tyr Cys Gly Asn
 115 120 125
 Pro Gln Asp Phe Phe Cys Lys Gln Trp Ser Cys Ile Thr Ser Asn Asp
 130 135 140
 35Gly Asn Trp Lys Trp Pro Val Ser Gln Gln Asp Arg Val Ser Tyr Ser
 145 150 155 160
 Phe Val Asn Asn Pro Thr Ser Tyr Asn Gln Phe Asn Tyr Gly His Gly
 165 170 175
 Arg Trp Lys Asp Trp Gln Gln Arg Val Gln Lys Asp Val Arg Asn Lys
 40 180 185 190

Gln Ile Ser Cys His Ser Leu Asp Leu Asp Tyr Leu Lys Ile Ser Phe
 195 200 205
 Thr Glu Lys Gly Lys Gln Glu Asn Ile Gln Lys Trp Val Asn Gly Ile
 210 215 220
 5Ser Trp Gly Ile Val Tyr Tyr Gly Gly Ser Gly Arg Lys Lys Gly Ser
 225 230 235 240
 Val Leu Thr Ile Arg Leu Arg Ile Glu Thr Gln Met Glu Pro Pro Val
 245 250 255
 Ala Ile Gly Pro Asn Lys Gly Leu Ala Glu Gln Gly Pro Pro Ile Gln
 10 260 265 270
 Glu Gln Arg Pro Ser Pro Asn Pro Ser Asp Tyr Asn Thr Thr Ser Gly
 275 280 285
 Ser Val Pro Thr Glu Pro Asn Ile Thr Ile Lys Thr Gly Ala Lys Leu
 290 295 300
 15Phe Ser Leu Ile Gln Gly Ala Phe Gln Ala Leu Asn Ser Thr Thr Pro
 305 310 315 320
 Glu Ala Thr Ser Ser Cys Trp Leu Cys Leu Ala Ser Gly Pro Pro Tyr
 325 330 335
 Tyr Glu Gly Met Ala Arg Gly Gly Lys Phe Asn Val Thr Lys Glu His
 20 340 345 350
 Arg Asp Gln Cys Thr Trp Gly Ser Gln Asn Lys Leu Thr Leu Thr Glu
 355 360 365
 Val Ser Gly Lys Gly Thr Cys Ile Gly Met Val Pro Pro Ser His Gln
 370 375 380
 25His Leu Cys Asn His Thr Glu Ala Phe Asn Arg Thr Ser Glu Ser Gln
 385 390 395 400
 Tyr Leu Val Pro Gly Tyr Asp Arg Trp Trp Ala Cys Asn Thr Gly Leu
 405 410 415
 Thr Pro Cys Val Ser Thr Leu Val Phe Asn Gln Thr Lys Asp Phe Cys
 30 420 425 430
 Val Met Val Gln Ile Val Pro Arg Val Tyr Tyr Tyr Pro Glu Lys Ala
 435 440 445
 Val Leu Asp Glu Tyr Asp Tyr Arg Tyr Asn Arg Pro Lys Arg Glu Pro
 450 455 460
 35Ile Ser Leu Thr Leu Ala Val Met Leu Gly Leu Gly Val Ala Ala Gly
 465 470 475 480
 Val Gly Thr Gly Thr Ala Ala Leu Ile Thr Gly Pro Gln Gln Leu Glu
 485 490 495
 Lys Gly Leu Ser Asn Leu His Arg Ile Val Thr Glu Asp Leu Gln Ala
 40 500 505 510

6

Leu Glu Lys Ser Val Ser Asn Leu Glu Glu Ser Leu Thr Ser Leu Ser
 515 520 525
 Glu Val Val Leu Gln Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Lys
 530 535 540
 5Glu Gly Gly Leu Cys Val Ala Leu Lys Glu Glu Cys Cys Phe Tyr Val
 545 550 555 560
 Asp His Ser Gly Ala Ile Arg Asp Ser Met Ser Lys Leu Arg Glu Arg
 565 570 575
 Leu Glu Arg Arg Arg Arg Glu Arg Glu Ala Asp Gln Gly Trp Phe Glu
 10 580 585 590
 Gly Trp Phe Asn Arg Ser Pro Trp Met Thr Thr Leu Leu Ser Ala Leu
 595 600 605
 Thr Gly Pro Leu Val Val Leu Leu Leu Leu Thr Val Gly Pro Cys
 610 615 620
 15Leu Ile Asn Arg Phe Val Ala Phe Val Arg Glu Arg Val Ser Ala Val
 625 630 635 640
 Gln Ile Met Val Leu Arg Gln Gln Tyr Gln Gly Leu Leu Ser Gln Gly
 645 650 655
 Glu Thr Asp Leu
 20 660

<210> 5

<211> 638

<212> PRT

25<213> Porcine endogenous retrovirus

<400> 5

Met His Pro Thr Leu Asn Arg Arg His Leu Pro Ile Arg Gly Gly Lys
 1 5 10 15
 30Pro Lys Arg Leu Lys Ile Pro Leu Ser Phe Ala Ser Ile Ala Trp Phe
 20 25 30
 Leu Thr Leu Ser Ile Thr Ser Gln Thr Asn Gly Met Arg Ile Gly Asp
 35 40 45
 Ser Leu Asn Ser His Lys Pro Leu Ser Leu Thr Trp Leu Ile Thr Asp
 35 50 55 60
 Ser Gly Thr Gly Ile Asn Ile Asn Asn Thr Gln Gly Glu Ala Pro Leu
 65 70 75 80
 Gly Thr Trp Trp Pro Asp Leu Tyr Val Cys Leu Arg Ser Val Ile Pro
 85 90 95
 40Ser Leu Thr Ser Pro Pro Asp Ile Leu His Ala His Gly Phe Tyr Val

	100		105		110
Cys Pro Gly	Pro Pro Asn Asn Gly Lys His Cys Gly Asn Pro Arg Asp				
	115		120		125
Phe Phe Cys Lys Gln Trp Asn Cys Val Thr Ser Asn Asp Gly Tyr Trp					
5	130		135		140
Lys Trp Pro Thr Ser Gln Gln Asp Arg Val Ser Phe Ser Tyr Val Asn					
145		150		155	160
Thr Tyr Thr Ser Ser Gly Gln Phe Asn Tyr Leu Thr Trp Ile Arg Thr					
	165		170		175
10Gly Ser Pro Lys Cys Ser Pro Ser Asp Leu Asp Tyr Leu Lys Ile Ser					
	180		185		190
Phe Thr Glu Lys Gly Lys Gln Glu Asn Ile Leu Lys Trp Val Asn Gly					
	195		200		205
Met Ser Trp Gly Met Val Tyr Tyr Gly Gly Ser Gly Lys Gln Pro Gly					
15	210		215		220
Ser Ile Leu Thr Ile Arg Leu Lys Ile Asn Gln Leu Glu Pro Pro Met					
225		230		235	240
Ala Ile Gly Pro Asn Thr Val Leu Thr Gly Gln Arg Pro Pro Thr Gln					
	245		250		255
20Gly Pro Gly Pro Ser Ser Asn Ile Thr Ser Gly Ser Asp Pro Thr Glu					
	260		265		270
Ser Ser Ser Thr Thr Lys Met Gly Ala Lys Leu Phe Ser Leu Ile Gln					
	275		280		285
Gly Ala Phe Gln Ala Leu Asn Ser Thr Thr Pro Glu Ala Thr Ser Ser					
25	290		295		300
Cys Trp Leu Cys Leu Ala Ser Gly Pro Pro Tyr Tyr Glu Gly Met Ala					
305		310		315	320
Arg Arg Gly Lys Phe Asn Val Thr Lys Glu His Arg Asp Gln Cys Thr					
	325		330		335
30Trp Gly Ser Gln Asn Lys Leu Thr Leu Thr Glu Val Ser Gly Lys Gly					
	340		345		350
Thr Cys Ile Gly Lys Val Pro Pro Ser His Gln His Leu Cys Asn His					
	355		360		365
Thr Glu Ala Phe Asn Gln Thr Ser Glu Ser Gln Tyr Leu Val Pro Gly					
35	370		375		380
Tyr Asp Arg Trp Trp Ala Cys Asn Thr Gly Leu Thr Pro Cys Val Ser					
385		390		395	400
Thr Leu Val Phe Asn Gln Thr Lys Asp Phe Cys Ile Met Val Gln Ile					
	405		410		415
40Val Pro Arg Val Tyr Tyr Tyr Pro Glu Lys Ala Ile Leu Asp Glu Tyr					

8

				420						425					430				
	Asp	Tyr	Arg	Asn	His	Arg	Gln	Lys	Arg	Glu	Pro	Ile	Ser	Leu	Thr	Leu			
				435					440					445					
	Ala	Val	Met	Leu	Gly	Leu	Gly	Val	Ala	Ala	Gly	Val	Gly	Thr	Gly	Thr			
5		450					455						460						
	Ala	Ala	Leu	Val	Thr	Gly	Pro	Gln	Gln	Leu	Glu	Thr	Gly	Leu	Ser	Asn			
	465					470					475					480			
	Leu	His	Arg	Ile	Val	Thr	Glu	Asp	Leu	Gln	Ala	Leu	Glu	Lys	Ser	Val			
				485						490					495				
10	Ser	Asn	Leu	Glu	Glu	Ser	Leu	Thr	Ser	Leu	Ser	Glu	Val	Val	Leu	Gln			
				500					505					510					
	Asn	Arg	Arg	Gly	Leu	Asp	Leu	Leu	Phe	Leu	Lys	Glu	Gly	Gly	Leu	Cys			
				515				520						525					
	Val	Ala	Leu	Lys	Glu	Glu	Cys	Cys	Phe	Tyr	Val	Asp	His	Ser	Gly	Ala			
15		530					535						540						
	Ile	Arg	Asp	Ser	Met	Asn	Lys	Leu	Arg	Glu	Arg	Leu	Glu	Lys	Arg	Arg			
	545					550					555					560			
	Arg	Glu	Lys	Glu	Thr	Thr	Gln	Gly	Trp	Phe	Glu	Gly	Trp	Phe	Asn	Arg			
				565						570					575				
20	Ser	Leu	Trp	Leu	Ala	Thr	Leu	Leu	Ser	Ala	Leu	Thr	Gly	Pro	Leu	Ile			
				580					585					590					
	Val	Leu	Leu	Leu	Leu	Thr	Val	Gly	Pro	Cys	Ile	Ile	Asn	Lys	Leu				
				595			600						605						
	Ile	Ala	Phe	Ile	Arg	Glu	Arg	Ile	Ser	Ala	Val	Gln	Ile	Met	Val	Leu			
25		610					615					620							
	Arg	Gln	Gln	Tyr	Gln	Ser	Pro	Ser	Ser	Arg	Glu	Ala	Gly	Arg					
	625					630					635								

<210> 6

30<211> 704

<212> DNA

<213> Porcine endogenous retrovirus

<400> 6

35	aattgaaagga	tgaaaatgca	acctgactct	ccagaaacc	agggaagttaa	taagaagctc	60
	taaatgccct	cgaaattccg	acctgtgttc	cttatggtaa	aagatcatac	ttttctgtgt	120
	tttaaaatct	cgcttctctg	ctgtcacaaa	ctttagtga	ggggaaaaac	aggccctgta	180
	gtatgtgcct	ctatgcttga	aacttcttga	aactgtctct	aactgcttgt	ttggctctgt	240
	taaacctctt	tgcataagat	aaaaagagga	gaactcaatt	gcacatcgca	ccccgaatag	300
40	atcggggtga	ccacaaaatt	tgtaaacaca	taatttqatg	acctaatact	tccccacc	360

9

cgaaacatgc gcaaatgtgt aactctaaaa caatttaaat taattggtcc acgaagcgcg 420
 ggctctcgaa gttttaaatt gactgggttg tgatatatttg aaatgatttg ttgttaaagc 480
 gggggtttg ttgtgaaccc cataaaagct gtcccgactc cacactcggg gccgcagtc 540
 tctacccctg cgtgggtgtac gactgtgggc cccagcgcgc ttggaataaa aatcctcttg 600
 5ctggttgcat caagaccgct tctcgtgagt gattaagggg agtcgccttt tccgagcctg 660
 gaggttcttt ttgctagtct tacatttggg ggctcgtccg ggat 704

<210> 7

<211> 633

10<212> DNA

<213> Porcine endogenous retrovirus

<400> 7

aatgaaagga tgaaaataca acctaagcta atgagaagct taaaattggt ctgaattcca 60
 15gagttgttct ctataggtta aaagattagg ttttttgctg ttttaaaata tgcggaagta 120
 aaataggccc tgagtacatg tctctaggca tgaaacttct tgaaactatt tgagataaca 180
 agaaaagggg gtttctaact gcttggttag ctctgtataa actgggttgc ccataaagat 240
 gttgaaatgt tgatacacat atcttggtga caacatgtct cccccacccc gaaacatcgc 300
 caaatgtgta actctaaaaa aatttaaatt aattgggtcca cgaagcgcgg gctctcgaag 360
 20ttttaaatg actgggttgt gatatttga aatgattggt ttgtaaagcg cgggctttgt 420
 tgtgaacccc ataaaagctg tcccgaactc acactcgggg ccgcagtcct ctaccctcgc 480
 tggtgtacg actgtgggccc ccagcgcgct tggaataaaa atcctcttgc tgtttgcac 540
 aagaccgctt ctctgtagtg attaaagggg gtcgcctttt ccgagccttg aggttctttt 600
 tgctgggtctt acatttgggg gctcgtcccg gat 633

25

<210> 8

<211> 20

<212> DNA

<213> Porcine endogenous retrovirus

30

<400> 8

tggaagatt ggcaacagcg 20

<210> 9

35<211> 20

<212> DNA

<213> Porcine endogenous retrovirus

<400> 9

40agtgatgtta ggctcagtg 20

10

<210> 10

<211> 20

<212> DNA

<213> Porcine endogenous retrovirus

5

<400> 10

ttctcctttg tcaattccgg

20

<210> 11

10<211> 20

<212> DNA

<213> Porcine endogenous retrovirus

<400> 11

15tacttttatcg ggtccactg

20

<210> 12

<211> 20

<212> DNA

20<213> Porcine endogenous retrovirus

<400> 12

ctgacctgga ttagaactgg

20

25<210> 13

<211> 20

<212> DNA

<213> Porcine endogenous retrovirus

30<400> 13

atgttagagg atggtcctgg

20

<210> 14

<211> 22

35<212> DNA

<213> Porcine endogenous retrovirus

<400> 14

acctcgagac tcggtggaag gg

22

40

11

<210> 15
 <211> 24
 <212> DNA
 <213> Porcine endogenous retrovirus
 5
 <400> 15
 ctgggtctcg ggagggttag gttg 24

 <210> 16
 10<211> 24
 <212> DNA
 <213> Porcine endogenous retrovirus

 <400> 16
 15acgtactgga ggagggtcac ctga 24

 <210> 17
 <211> 24
 <212> DNA
 20<213> Porcine endogenous retrovirus

 <400> 17
 gtcccgaaacc cttataacct cttg 24

 25<210> 18
 <211> 1980
 <212> DNA
 <213> Porcine endogenous retrovirus

 30<400> 18
 atgcattccca cgttaagccg gcgccacctc cggattcggg gtggaaagcc gaaaagactg 60
 aaaatccccct taagcttcgc ctccatcgcg tgggttcctta ctctgtcaat aactcctcaa 120
 gttaatggta aacgccttgt ggacagcccg aactcccata aacccttate tctcacctgg 180
 ttacttactg actccggtac aggtattaat attaacagca ctcaagggga ggctcccttg 240
 35gggacctggg ggcctgaatt atatgtctgc ctccgatcag taatccctgg tctcaatgac 300
 caggccacac cccccgatgt actccgtgct tacggggttt acgtttgccc agggccccc 360
 aataatgaag aatatgtggg aaatccctcag gatttctttt gcaagcaatg gagctgcgta 420
 acttctaattg atgggaattg gaaatggcca gtctctcagc aagacagagt aagttactct 480
 ttgttaaca atcctaccag ttataatcaa tttaattatg gccatgggag atggaaagat 540
 40tggaacacgc ggggtacaaa agatgtacga aataagcaaa taagctgtca ttcgttagac 600

ctgattact taaaaataag ttctactgaa aaaggaaaac aagaaaatat tcaaaagtgg 660
 gtaaatggta tgccttgggg aatagtgtac tatagaggct ctgggagaaa gaaaggatct 720
 gtctgacta ttccgctcag aatagaaact cagatggaaac ctccgggtgc tataggacca 780
 aataagggtt tggccgaaca aggacctcca atccaagaac agaggccatc tcctaaccctc 840
 5ctctgattaca atacaacctc tggatcagtc cccactgagc ctaacatcac tattaanaaa 900
 ggggcgaaac tttttaacct catccaggga gcttttcaag ctcttaactc cagactcca 960
 gaggtacct ctctctgttg gctttgctta gcttcgggcc cacttacta tgagggaatg 1020
 gctagaggag ggaattcaa tgtgacaaag gaacatagag accaatgtac atggggatcc 1080
 caaataagc ttaccttac tgaggtttct ggaaaaggca cctgcatagg gatgggtccc 1140
 10ccatcccacc aacacctttg taaccacact gaagccttta atcgaaacctc tgagagtcag 1200
 tatctggtag ctggttatga cagggtgttg gcatgtaata ctggaataac cccttgtgtt 1260
 tccaccttgg ttttcaacca aactaaagac ttttgctta tggtcctaat tgcctccctg 1320
 gtgtactact atccccaaaa agcagtcctt gatgaatatg actatagata taatcggcca 1380
 aaaagagagc ccataacctc gacctagct gtaatgctcg gattgggagt ggctgcaggc 1440
 15gtgggaacag gaacgggtgc cctaatcaca ggaccgcaac agctggagaa aggaacttagt 1500
 aaactacatc gaattgtaac ggaaaatctc caagccctag aaaaatctgt cagtaacctg 1560
 gaggaatccc taacctcctt atctgaagtg gttctacaga acagaagggg gttagatctg 1620
 ttatttctaa aagaaggagg attatgtgta gccttaagg aggaatgctg tttttatgtg 1680
 gatcattcag gggccatcag agactccatg aacaagctta gagaagggtt ggagaagcgt 1740
 20cgaaaggaaa aggaactac tcaagggttg tttgagggat ggttcaacag gtctccttgg 1800
 ttggtacccc tactttctgc tttaacagga ccttaatatg tctctcctct gttactcaca 1860
 ttggggcatc gtattattaa caagttaatt gccttcatta gagaacgaat aagtgcagtc 1920
 cagatcatgg tacttagaca acagtaccaa agcccgctca gcagagaagc tggccgctag 1980

25<210> 19

<211> 7362

<212> DNA

<213> Porcine endogenous retrovirus

30<400> 19

tacttcttgg ggaagacctt gggctgctaa ctgggtcttg gctggctccta gtgaaaggat 60
 gaaaatgcac cctgactctc ccagaaccca ggaagttaat aagaagctct aaataatgaa 120
 aggatgaaaa tgcaacctga ctctcccaga acccaggaag ttaataagaa gctctaaatg 180
 cctcgaatt ccagacctg ttcctatag gtaaaagatc atactttttg ctgttttagg 240
 35gcttgctttc tgctctgtac aaaactttgt ggaaggggaa aaacaggccc ctgagtattg 300
 gcctctatgc ttgaaaattc ttgaaactgc tcctaactgc ttgtttggct ttgtgtaaac 360
 tgcttgcata agataaaaa aggagaagtc aattgcctaa cggaccccg taagatcggg 420
 tgtaccacaa aatgttgaaa cacatatctt ggtgacaaca tgtctcccc accccgaaac 480
 atgcgcaaat gtgtaactct aaacaattt aaattaattg gtccacgaag cgcggtctct 540
 40cgaggtttta aattgactgg tttgtgatat ttgaaatga ttggtttgta aagcgcgggc 600

ttgtttgtga	acccccataaa	agctgtcccc	actccacact	cggggcccgca	gtcctctacc	660
cttgogtggg	gtacgactgt	gggccccagc	cgccttgga	taaaaatcct	cttgctgttt	720
gcatcaagac	cgcttctcgt	gagtgattaa	ggggagtcgc	cttttccgag	cttgagagtt	780
ctttttgcta	gtcttacatt	tgggggctcg	tcggggtatc	gtcgcggcca	ccccatacac	840
5ccgagaaccg	acttgagggt	aaaaaggatc	ctctttttaa	ctgtgatgca	tgaccggcc	900
ggcgtctctg	ttctgagtgt	ctgtttttag	tgggtgcgcg	tttcggtttg	cagctgtcct	960
ctcagaccgt	aaggactggg	ggactgtgat	cagcagacgt	gctaggagga	tcacaggctg	1020
ccaccctggg	ggagcggccg	ggaggtgggg	agagccaggg	acgcctgggt	gtctcctctc	1080
gtcgttcaga	ggaccgagtt	ctgttgttga	agcgaaagct	ccccctccg	cggccgtccg	1140
10actcttttgc	ctgcttggtg	aagacgcgga	cgggtcgcgt	gtgtctggat	ctgttggttt	1200
ctgtttttgt	tgcttttgtc	ttgtgcgtcc	ttgtctacag	ttttaaatatg	ggacagacgg	1260
tgacgacccc	tcttagtttg	actctcgacc	attggactga	agttaaatcc	agggctcata	1320
atttgcagtt	tcaggttaag	aagggaacct	ggcagacttt	ctgtgtctct	gaatggccga	1380
cattcgatgt	tggatggcca	tcagagggga	cccttaattc	tgagattatc	ctggctgtta	1440
15aagcaattat	ttttcagact	ggaccggcgt	ctcatcccaa	tcaggagccc	tatacctcta	1500
cgtggcaaga	tttggcagag	gacccctccg	catgggttaa	accttggctg	aataagccaa	1560
gaaagccagg	tcccgaatt	ctggctcttg	gagagaaaaa	caaacactcg	cgtgaaaaag	1620
tcaagccctc	tcctcatatc	taccocgaga	ttgaggagcc	gccggcttgg	cgggaacccc	1680
aatctgttcc	cccacccctc	tatctggcac	agggtgctgc	gaggggaccc	tctgccctc	1740
20ctggagctcc	ggcgggtggg	ggacctgctg	cagggaactcg	gagccggagg	ggccgccccc	1800
cggagccggg	agacgagatc	gcgacattac	cgctgcgcac	gtacggccct	cccacaccgg	1860
ggggccaatt	gcagccctcc	cagtattggc	ccctttcttc	tcgagatctc	tataattgga	1920
aaactaacca	tcaccttttc	tcggaggatc	cccaacgcct	cacggggttg	gtggagtccc	1980
ttatgttctc	tcaccagcct	acttgggatg	attgtcaaca	gctgctgcag	acactcttca	2040
25caaccgagga	gcgagagaga	attctgttag	aggctagaaa	aaatgttctc	ggggccgacg	2100
ggcgaccacc	gcagttgcaa	aatgagattg	acatgggatt	tccttgcact	cgccccgggt	2160
gggactacaa	ctacgtgaa	ggtagggaga	gcttgaaaaa	ctatcgccag	gctctgggtg	2220
cgggtctccg	gggcgcctca	agacggccca	ctaatttggc	taaggttaaga	gaagtgatgc	2280
agggaccgaa	tgaacccccc	tctgttttcc	ttgagaggct	cttggaaagc	ttcaggcggt	2340
30acaccccttt	tgatccccacc	tcagaggccc	aaaaagccct	agtggccttg	gcctttatat	2400
gacagtcagc	cttggatatt	agaaaagaagc	ttcagagact	ggaagggtta	caggaggctg	2460
agttactgta	cttagtgaag	gaggcagaga	aagtataatta	caaaaggag	acagaagaag	2520
aaagggaaca	aagaaaagag	agagaagag	aggaaaggga	ggaagacgt	aataaacggc	2580
aagagaagaa	tttgactaag	atcttggctg	cagtgggtga	agggaaaagc	aatacggaaa	2640
35gagagagaga	tttttaggaaa	attaggtcag	gccctagaca	gtcagggaac	ctgggcaata	2700
gagccact	cgacaagagc	caatgtgcat	attgtaaga	aaaaggacac	tgggcaagga	2760
actgcccaa	gaagggaac	aaaggactga	aggtcttagc	cttgaaagaa	gataaagact	2820
aggggaagacg	gggttcggac	ccccctcccc	agcccgaggt	aactttgaag	gtggaggggc	2880
aaccagttga	gttcctggtt	gataccggag	cgaaacattc	agtgtacta	cagccattag	2940
40gaaaactaaa	agataaaaaa	tcctgggtga	tgggtgccac	agggcaacaa	cagtatccat	3000

ggactaccgg	aagaacagtt	gacttggggag	tgggacgggt	aaccactcg	tttctggtca	3060
tacctgagtg	cccagcacc	ctcttaggta	gagacttatt	gaccaagatg	ggagcacaaa	3120
ttctctttga	acaagggaaa	ccagaagtgt	ctgc aaataa	caaaccatc	actgtgttga	3180
ccctccaatt	agatgacgaa	tatcgactat	actctcccc	agtaaagcct	gatcaaaata	3240
5tacaattctg	gttggaaacag	tttccccaa	cctgggcaga	aaccgcagg	atgggtttgg	3300
caaagcaagt	tccccacaa	gttattcaac	tgaagccag	tgccacacca	gtgtcagta	3360
gacagtacc	cttgagtaaa	gaagctcaag	aaggaattcg	gccgcattgc	caaagattaa	3420
tccaacaggg	catcctagtt	cctgtccaat	ctccctggaa	tactccccct	ctacccggtta	3480
gaaagcctgg	gactaatgac	tatcgaccag	tacaggactt	gagagaggtc	aataaacggg	3540
10tgaggatata	acaccaca	gtccccgaac	cttataacct	cttgtgtgct	ctcccccccc	3600
aacggagctg	gtatacagta	ttggacttaa	aggatgcctt	tttctgctg	agattacacc	3660
ccactagcca	accacttttt	gccttcgaat	ggagagatcc	aggtaacggga	agaacccggg	3720
agctcaactg	gaccgcagct	ccccaaaggt	tcaagaactc	cccgaccatc	tttgacgaag	3780
ccctacacag	agacctggcc	aacttcagga	tccaacacce	tcaggtgacc	ctcctccagt	3840
15acgtggatga	cctgtctctg	gcgggagcca	ccaaacagga	ctgcttagaa	ggcacgaagg	3900
cactactgct	ggaattgtct	gacctaggct	acagagcctc	tgctaagaag	gccagatttt	3960
gcaggagaga	ggttaacatac	ttggggtaca	gtttgcggga	cgggcagcga	tgggtgacgg	4020
aggcaccgaa	gaaaactgta	gtccagatac	cgcccccaac	cacagccaaa	caagtggagag	4080
agtttttggg	gacagctgga	ttttgcagac	tgtggatccc	ggggtttgcc	accttagcag	4140
20ccccactcta	ccactaacc	aaagaaaaag	gggaattctc	ctgggctcct	gagcaccaga	4200
aggcatttga	tgtatcaaa	aaggccctcg	tgagcgacc	tgtcttgccc	ctccctgacg	4260
taactaaacc	ctttaccctt	tatgtggatg	agcgtaaggg	agtagccggg	ggagtttttaa	4320
cccaaactct	aggaccatgg	aggagacctg	ttgcctacct	gtcaaaagaag	ctcgatcctg	4380
tagccagtgg	ttggcccgta	tgccctgaag	ctatcgacg	tgtggccata	ctggccaagg	4440
25acgtgacaa	attgactttg	ggacagaata	taactgtaat	agcccccat	gcgttgagga	4500
acatcgcttg	gcagccccca	gaccgatgga	tgaccaacgc	ccgatgacc	cactatcaaa	4560
gcctgctctc	cacagagagg	gtcacgtttc	ctccaccagc	cgctctcaac	ctcgccaetc	4620
ttctgcctga	agagactgat	gaaccagtga	ctcatgattg	ccatcaacta	ttgattgagg	4680
agactgggg	ccgcaaggac	cttacagaca	taccgctgac	tggagaagtg	ttaacctggt	4740
30tcactgacgg	aagcagctat	gtggtggaag	gtaagaggat	ggctggggcg	gcggtggttg	4800
acgggacccg	cacgatcttg	gccagcagcc	tgccggaagg	aacttcagca	caaaaggctg	4860
agctcatggc	cctcacgcaa	gctttgcggc	tgcccggaag	gaaatccata	aacattttata	4920
cagacagcag	gtatgccttt	gcgactgcac	acgtacacgg	ggccatctat	aagcaaaagg	4980
ggtgtcctac	ctcagcaggg	agggaataa	agaacaaaga	ggaaattcta	agcctattag	5040
35aagcettaca	tttgccaaaa	aggctageta	ttatacactg	tcctggacat	cagaaagcca	5100
aagatcccat	atccagatgg	aaccagatgg	ctgaccgggt	tgccaaagcag	gcagccaggg	5160
gtgttaacct	ctgtcctatg	atagaaacac	ccaaagcccc	agaacccgca	cgaactata	5220
ccctagaaga	ctggcaagag	ataaaaaaga	tagaccaggt	ctctgagact	ccggaaggga	5280
cctgctatac	ctcagatggg	aaggaaatcc	tgccccacaa	agaagggtta	gaatatgtcc	5340
40aacagataca	tcgtctaacc	caccttaggaa	ctaaacacct	gcagcagttg	gtcagaacat	5400

15

ctccttatca	tgttctgagg	ctaccaggag	tggctgattc	ggtgggtcaaa	cactgtgtgc	5460
cctggccagct	gggtaaatgct	aatccttcca	gaatacctcc	aggaaagaga	ctaaggggaa	5520
gccacccagg	cgctcactgg	gaagtggact	tcactgaggt	aaagccggct	aaatacggaa	5580
acaaatatct	attggttttt	gtagacacct	tttcaggatg	ggtagaggct	tatctacta	5640
5agaaagagac	ttcaaccgtg	gtggctaaaa	aaatactgga	ggaaattttt	ccaagatttg	5700
gaatacctaa	ggtaaatagg	tcagacaatg	gtccagcttt	cggtgcccg	gtaagtcagg	5760
gactggccaa	gatattgggg	attgatgtga	aactgcattg	tgcatacaga	ccccaaagct	5820
caggacaggt	agagaggatg	aatagaacca	ttaaagagac	ccttactaaa	ttgaccgagg	5880
agactggcgt	taagtattgg	atagctctcc	tgccctttgt	actttttagg	gttaggaaca	5940
10ccccctggaca	gtttgggctg	accccttatg	aattactcta	cgggggaccc	ccccattgg	6000
tagaaaattgc	ttccgtacat	agtgctgacg	tgctgctttc	ccagcccttg	ttctctaggc	6060
tcaaggcact	tgagtggggt	agacaaagag	cgaggaggca	actccgggag	gcctactcag	6120
gaggagggaga	cttgacagat	ccacatcggt	tccaagtggg	agattcagtc	tcaggttagac	6180
gccaccgtgc	aggaaacctc	gagactcggt	ggaagggccc	ttatcacgta	cttttgacca	6240
15caccaacggc	tgtgaaagtc	gaaggaaatct	ccacctggat	ccatgcaccc	cacgttaagc	6300
cgggccacc	tcocgattcg	gggtggaaag	ccgaaaagac	tgaaaaatccc	cttaagcttc	6360
gctccatcg	cggtggtcct	tactctgtca	ataactcctc	aagttaatgg	ttaacgcctt	6420
gtggacagcc	cgaactccca	ttaaccttta	tctctacact	ggttacttac	tgactccggg	6480
acaggtatta	atattaacag	cactcaaggg	gaggtccctc	tggggacctg	gtggcctgaa	6540
20ttatatgt	ctgcctcgatc	agtaatccct	ggtctcaatg	accaggccac	acccccgat	6600
gtactccgtg	cttacggggt	ttacgtttgc	ccaggacccc	caaataatga	agaatatgtg	6660
ggaaatcctc	aggatttcct	ttgcaagcaa	tggagctcgc	taactttcaa	tgatgggaat	6720
tggaatggc	cagttcttca	gcaagacaga	gtaagttact	cttttgtaa	caatccctacc	6780
agttataatc	aatttaatta	tgcccatggg	agatggaaag	attggcaaca	gcgggtacaa	6840
25aaagatgtac	gaaataagca	aataagctgt	cattcgtag	acctagatta	cttaaaaaata	6900
agtttctactg	aaaaaggaaa	acaagaaaaa	attcaaaagt	gggtaaatgg	tatgtcttgg	6960
ggaaatgctg	actatggagg	ctctgggaga	aagaaaggat	ctgttctgac	tatgccctc	7020
agaatagaaa	ctcagatgga	acctccgggt	gctataggac	caaataaggg	tttggccgaa	7080
caaggacctc	caatccaaga	acagaggcca	tctcctaacc	cctctgatta	caatacaacc	7140
30ctcgatcag	tcoccatgga	gcctaaccac	actattaaaa	caggggcgaa	actttttaac	7200
ctcatccagg	gagcttttca	agctcttaac	tccacgactc	cagaggctac	ctcttcttgc	7260
tgctcttgcg	tagcttcggg	ccaccttac	tatgagggaa	tggctagagg	agggaatttc	7320
aatgtgacaa	aggaaacatg	agaccaatgt	acatggggat	cc		7382

35

<210> 20

<211> 4402

<212> DNA

40<213> Porcine endogenous retrovirus

<400> 20						
gatcttggct	gcagctgggtg	aagggaaaaag	caatacggaa	agagagagag	atttttaggaa	60
aattaggtca	ggccctagac	agtcagggaa	cctgggcaat	aggacccacc	tcgacaagga	120
ccaatgtgca	tattgtaaa	aaaaaggaca	ctgggcaagg	aactgcccc	agaagggaaa	180
5caaaaggactg	aaggtcttag	ctctggaaga	agataaagac	taggggaagac	gggggtcggga	240
ccccctcccc	gagcccaggg	taactttgaa	ggtggagggg	caaccaggtg	agttcctcgt	300
tgataccgga	gcgaaaacatt	cagtgtctact	acagccatta	ggaaaaactaa	aagataaaaa	360
atcctgggtg	atgggtgccca	cagggcaaca	acagtatcca	tggactacc	gaagaacagt	420
tgacttggga	gtgggacggg	taaccactc	gtttctggtc	atacctgagt	gcccagcacc	480
10cctcttaggt	agagactatt	tgaccaagat	gggagacaaa	atttcttttg	aacaagggaa	540
accagaagtg	tctgcaataa	acaaacctat	cactgtgttg	accctccaat	tagatgacga	600
atatcgacta	tactctcccc	tagtaaaagc	tgatcaaaat	atacaattct	ggttggaaca	660
gtttcccaa	gcctggcgag	aaaccgcagg	gatgggtttg	gcaaaagcaag	ttccccaca	720
agttattcaa	ctgaaggcca	gtgccacacc	agtgtcagtc	agacagtacc	ccttgagtaa	780
15agaagctcaa	gaaggaaattc	ggccgcgatg	ccaaagatta	atccaacagg	gcactcctagt	840
tcctgtccaa	tctccctgga	atactccctt	gctaccgggt	agaaagcctg	ggactaatga	900
ctatcgacca	gtacaggact	tgagagaggt	caataaacgg	gtgcaggata	tacacccaac	960
agtcccgaac	ccttataaac	tcttgtgtgc	tctcccaccc	caacggagct	ggtatacagt	1020
attggactta	aaggatgcct	ttttctgcct	gagattacac	cccactagcc	aaccactttt	1080
20tgccctcgaa	tggagagatc	caggtacggg	aagaaccggg	cagctcacct	ggaccgcact	1140
gccccaaagg	ttcaagaact	ccccgaccat	ctttgacgaa	gccctacaca	gagacctggc	1200
caacttcagg	atccaacacc	ctcaggtgac	cctcctccag	tacgtggatg	acctgcttct	1260
ggcgggagcc	accaaaccagg	actgcttaga	aggcacgaag	gcactactgc	tggaaattgtc	1320
tgacctaggc	tacagagcct	ctgctaagaa	ggcccagatt	tgcaggagag	aggtaacata	1380
25cctgggttac	agtttgcggg	acgggcagcg	atggctgacg	gaggcacgga	agaaaactgt	1440
agtccagata	ccggccccc	ccacagccaa	acaagtgaga	gagttttttg	ggacagctgg	1500
attttgcaga	ctgtggatcc	cgggggtttg	gaccttagca	gccccactct	accactaac	1560
caaaagaaaa	ggggaattct	cctgggctcc	tgagcaccag	aaggcatttg	atgctatcaa	1620
aaaggccctg	ctgagcgcac	ctgctctggc	cctccctgac	gtaactaaac	cctttaccct	1680
30ttatgtggat	gagcgtaaag	gagtagcccg	gggagtttta	acccaaacct	taggaccatg	1740
gaggagacct	gttgccctacc	tgtcaaaaga	gctcgatcct	gtagccagtg	gttggcccg	1800
atgcctgaag	gctatcgca	ctgtggccat	actggtcaag	gacgtgaca	aattgacttt	1860
gggacagaat	ataactgtaa	tagccccca	tgcgttggag	aacatcgttc	ggcagcccc	1920
agaccgatgg	atgaccaacg	cccgcatgac	ccactatcaa	agcctgcttc	tcacagagag	1980
35ggtcacgttc	gctccaccag	ccgctctcaa	ccctgccact	cttctgcctg	aagagactga	2040
tgaaccagtg	atcatgatt	gccatcaact	attgattgag	gagactgggg	tccgcaaggga	2100
ccttacagac	ataccgctga	ctggagaagt	gttaacctgg	ttcactgacg	gaagcagcta	2160
tgtgttgtaa	ggtaagagga	tggctggggc	ggcggtggtg	gacgggaccc	gcacgatctg	2220
ggccagcagc	ctgcgggaag	gaacttcagc	acaaaaggct	gagctcatgg	ccctcacgca	2280
40agctttgcgg	ctggccgaag	ggaaatccat	aaacatttat	acagacagca	ggtatgcctt	2340

tgcgactgca cacgtacacg gggccatcta taagcaaaagg gggttgctta cctcagcagg 2400
 gagggaaata aagaacaaag aggaattctc aagcctatta gaagccttac atttgccaaa 2460
 aaggctagct attatacact gtcctggaca tcagaaagcc aaagatccca tatccagagg 2520
 gaaccagatg gctgaccggg ttgccaagca ggcagcccag ggtgttaacc tctgcctat 2580
 5gatagaaca cccaaagccc cagaaccogg acgacagtac accctagaag actggcaaga 2640
 gataaaaaag atagaccagt tctctgagac tccggaaggg acctgtcata cctcagatgg 2700
 gaaggaaatc ctgcccacaca aagaagggtt agaatatgtc caacagatag atcgtctaac 2760
 ccacctagga actaacacc tgcagcagtt ggtcagaaca tctccttacc atgttctgag 2820
 gctaccagga gtggctgatt cgggtgtcaa acactgtgtg cctgtccagc tggtaaatgc 2880
 10taatccttcc agaatacctc caggaaagag actaagggga agccaccagg gcgctcactg 2940
 ggaagtggac ttactgagg taaagccggc taaatacggg aacaatatc tattggtttt 3000
 tgtagacacc ttttcaggat gggtagaggc ttatcctact aagaaagaga ctccaaccgt 3060
 ggtggctaag aaaatactgg aggaattttt tccaagattt ggaataccta aggtaataagg 3120
 gtcagacaat ggtccagctt tcgttgccca ggtaagtcag ggaactggcc agatattggg 3180
 15gattgattgg aaactgcatt gtgcatacag accccaaagc tcaggacagg tagagaggat 3240
 gaatagaacc attaaagaga cccttactaa attgaccggc gagactggcg ttaatgattg 3300
 gatagctctc ctgccctttg tgcttttttag ggttaggaac acccctggag agtltgggct 3360
 gacccctat gaattactct acgggggacc ccccccattg gtgaaattg cttccgtaca 3420
 tagtgctgac gtgctgtttt cccagccttt gttctctagg ctcaaggcac ttgagtgggt 3480
 20gagacaacga gcgtggaggc aactccggga ggccactca ggaggaggag acttgagat 3540
 cccacatcgt ttccaagtgg gagattcagt ctacgttaga cgccaccgtg caggaaacct 3600
 cgagactcgg tgggaaggcc cttatcacgt acttttgacc acaccaacgg ctgtgaaagt 3660
 cgaaggaatc tccacctgga tccatgcac ccacgttaag ccggcgccac ctcccgattc 3720
 ggggtggaaa gccgaaaaga ctgaaaatcc ccttaagctt cgctccatc gcgtggttcc 3780
 25ttactctgtc aataactcct caagttaatg gtaaacgcct tgtggacagc ccgaactccc 3840
 ataaacccct atctctcacc tggttactta ctgactccgg tacaggtatt aatattaaca 3900
 gcactcaagg ggaggctccc ttggggacct ggtggcctga attatatgtc tgccctcgat 3960
 cagtaatccc tggttctaat gaccaggcca cacccccga tgtactccgt gcttacgggt 4020
 tttacgtttg cccaggacc ccaataatg aagaatatg tggaaatcct caggatttct 4080
 30tttgcagca atggagctgc gtaacttcta atgatgggaa ttggaaatgg ccagctctc 4140
 agcaagacag agtaagttac tctttgtta acaatccact cagttataat caatttaatt 4200
 atggccatgg gagatggaaa gattggcaac agcgggtaca aaaagatga cgaataaagc 4260
 aaataagctg tcattcgtta gacctagatt acttaaaat aagtttcaat gaaaaaggaa 4320
 aacaagaaaa tattcaaaag tgggtaaatg gtatgtcttg gggaatagtg tactatggag 4380
 35gctctgggag aaagaaagga tc 4402

<210> 21

<211> 6076

<212> DNA

40<213> Porcine endogenous retrovirus

<400>	21					
ccccagagga	ggtgagaggc	tgtggcatag	ggaggcctcc	ctggaggagg	ttgaaaggat	60
gaaaatgc	ccctgactctc	ccagaaccca	ggaagttaat	aagaagctct	aaatgccctc	120
gaattccaga	ccctgttccc	tataggtaaa	agatcatact	ttttgctgtt	ttagggcttg	180
5ctttctgctc	tgtacaaaac	tttgtggaag	gggaaaaaca	ggccctctag	tatgtgccct	240
tatgcttgaa	acttcttgaa	actgctccta	actgcttggt	tggtctctgt	aaacctgctt	300
gcataagata	aaaagaggag	aagtcaattg	cctaacggac	cccagtaaga	tcgggtgtac	360
cacaaaatgt	tgaaacacat	atcttggtga	caacatgtct	ccccacccc	gaaacatgcy	420
caaatgtgta	actctaaaa	aatttaaatt	aattggtcca	cgaagcgccg	gctctcgaag	480
10ttttaaattg	actggtttgt	gatattttga	aatgattggt	ttgtaaacg	cgggctttgt	540
tggtgaacccc	ataaaagctg	tcccgaactc	acactcgggg	cgcgagctct	ctacccctgc	600
gtggtgtacg	actgtgggcc	ccagcgcgct	tggaaataaaa	atcctcttgc	tgtttgatc	660
aagaccgcct	ctcgtgagg	attaagggga	gtcgcctttt	ccgagcctgg	aggttctttt	720
tgctagtctt	acatttgggg	gctcgtccgg	gatctgtcgc	ggccacccct	aacacccgag	780
15aacgcagctg	gaggtaaaaa	ggatcctctt	tttaacgtgt	atgcattgac	cggccggcgt	840
ctctgttctg	agtgtctgtt	ttcagtggtg	cgcgctttcg	gtttgcagct	gtcctctcag	900
accgtaagga	ctgggggact	gtgatcagca	gacgtgctag	gaggatcaca	ggctgccacc	960
ctgggggagc	cccgcggagg	tgggggagag	caggggacgc	tggttggtctc	cttctgtcgg	1020
tcagaggacc	gagttctgtt	gttgaagcga	aagcttcccc	ctccgcggcc	gtccgactct	1080
20tttgcctgct	tgtggaagac	gcggacgggt	cgctgtgtgc	tggaatctgt	ggtttctgtt	1140
ttgtgtgtct	ttgtcttggt	cgctctgttc	tacagtttta	atatgggaca	gacggtgacg	1200
accctcttta	gtttgactct	cgaccattgg	actgaagtta	aatccagggc	tcataatttg	1260
tcagttcagg	ttaagaaggg	accttgccag	actttctgtg	tctctgaatg	gccacatttc	1320
gatgttggaat	ggccatcaga	ggggaccttt	aattctgaga	ttatcctggc	tgttaaagca	1380
25attatttttc	agactggacc	cggctctcat	cccaatcagg	agccctatat	ccttacgtgg	1440
caagattttg	cagaggatcc	tccgccatgg	gttaaaccct	ggctgaataa	gccaaagaaa	1500
ccaggtcccc	gaattctggc	tcttgagag	aaaaacaaca	actcggctga	aaaagtcaag	1560
ccctctctct	atatctaccc	cgagattgag	gagccgcggg	cttgcccgga	accccaatct	1620
gttccccacc	ccccttatct	ggcacagggt	gctgcgaggg	gacctctctg	ccctcttgga	1680
30gctccggcgg	tggaggggacc	tgctgcaggg	actcggagcc	ggaggggcgc	caccccgagg	1740
ggcacagacg	agatcgogac	attaccgctg	cgcacgtacg	gccctcccac	accggggggc	1800
caattgcagc	ccctccagta	ttggcccttt	tcttctgcag	atctctataa	ttggaaaact	1860
aaccatcccc	ctttctcgga	ggatccccaa	cgctctacgg	ggttggtgga	gtcccttatg	1920
ttctctcacc	agcctacttg	ggatgattgt	caacagctgc	tgcacagact	cttcacaacc	1980
35gaggagcgag	agagaattct	gttagaggct	agaaaaaatg	ttcctggggc	cgacggggcg	2040
ccccgcagct	tgcaaaatga	gattgacatg	ggatttcccc	tgaactcggc	cgggtgggac	2100
tacaacacgg	ctgaaggtag	ggagagcttg	aaaatctatc	gccaggetct	ggtggcgggt	2160
ctccggggcg	cctcaagacg	gcccactaat	ttggctaagg	taagagaagt	gatgcaggga	2220
cgaatgaac	ccccctctgt	tttcttgtag	aggtctcttg	aagccttcag	gcggtacacc	2280
40ccttttgatc	ccacctcaga	ggcccaaaaa	gcctcaatgc	ctttggcctt	tataagacag	2340

tcagccttgg	atattagaaa	gaagcttcag	agactggaag	ggttacagga	ggctgagtta	2400
cgtgatctag	tgaaggaggc	agagaaagta	tattacaaaa	gggagacaga	agaagaaaag	2460
gaacaaagaa	aagagagaga	aagagaggaa	agggagggaaa	gacgtaataa	acggcaagag	2520
aagaatttga	ctaagatctt	ggctgcagtg	gttgaaggga	aaagcaatac	ggaaagagag	2580
5agagatttta	ggaaaattag	gtcaggccct	agacagtcag	ggaacctggg	caataggacc	2640
ccactcgaca	aggaccaatg	tgcatattgt	aaagaaaaag	gacactgggc	aaggaaactgc	2700
cccaagaagg	gaaacaaagg	actgaaggtc	ttagctctcg	aagaagataa	agactaggga	2760
agaacggggt	cggaccccc	ccccgagccc	agggtaacct	tgaaggtgga	ggggcaacca	2820
gttgggttcc	tggttgatac	cggagcgaaa	cattcagtcg	tactacagcc	attaggaaaa	2880
10ctaaaagata	aaaaatcctg	ggtgatgggt	gccacagggc	aacaacagta	tccatggact	2940
acccgaagaa	cagttgactt	gggagtgggg	cgggtaaccc	actcgtttct	ggtcatacct	3000
gagtgcccag	cacccctctt	aggtagagac	ttattgacca	agatgggagc	acaaatttct	3060
tttgacaacg	ggaaaccaga	agtgtctgca	aataacaaac	ctatcactgt	gttagacctc	3120
caattagatg	acgaatatcg	actatactct	ccccagtaga	agcctgatca	aaatatacaa	3180
15ttctgggttg	aacagtttcc	ccaagcctgg	gcgaaaacgc	cagggatggg	tttgcaaaag	3240
caagttcccc	cacaagttaa	tcaactgaag	gccagtgcca	caccagtgtc	agtcagacag	3300
taccctctga	gtaaaagaag	tcaagaaggg	attcgccgcg	atgtccaaaag	attaatccaa	3360
cagggcatcc	tagttcctgt	ccaatctccc	tggaaatact	ccctgtctac	ggttagaaaag	3420
cttgggacta	atgactatcg	accagtcacg	gacttgagag	aggtcaataa	acgggtgcag	3480
20gatatacacc	caacagtcct	gaacctctat	aacctcttgt	gtgctctccc	accccaacgg	3540
agctgggtata	cagttattga	cttaaaggat	gcctttttct	gcctgagatt	acaccccatc	3600
agccaaaccac	tttttgcctt	cgaatggaga	gatccaggta	cgggaagaac	cgggcagctc	3660
acctggacc	gactgcccga	aggggttcaag	aactccccga	ccatctttga	cgaagcccta	3720
cacagagacc	tggccaactt	caggatccaa	caccctcagg	tgacctctct	ccagtacgtg	3780
25gatagacctg	ttctggcggg	agccaccaaa	caggactgct	tagaaggcac	gaaggcacta	3840
ctgctggaat	tgtctgacct	aggtacaga	gcctctgcta	agaaggccca	gatttgacgg	3900
agagaggtta	catactggg	gtacagtttg	cgggacgggc	agcgatgctg	cagcgaggga	3960
cggaaagaaa	ctgtagtcca	gataccggcc	ccaaccacag	ccaacaagt	gagagagttt	4020
ttggggacag	ctggattttg	cagactgtgg	atcccggggt	ttgcagacct	agcagcccca	4080
30ctctaccacc	taaccaaaga	aaaaggggaa	ttctcctggg	ctcctgagca	ccagaaggga	4140
tttgatgcta	tcaaaaaggc	cctgctgagc	gcacctgtct	tggccctccc	tgacgttaact	4200
aaacctttta	cccttatgtg	ggatgagcgt	aaggagtag	ccggggaggt	tttaacccaa	4260
actctaggac	catggaggag	acctgttgcc	tacctgtcaa	agaagctcga	tcctgtagcc	4320
agtgggtggc	ccgtatgcct	gaaggctatc	gcagctgtgg	ccatactggt	caaggacgct	4380
35gacaaattga	ctttgggaca	gaatataact	gtaataagcc	accatgcytt	ggagaaatc	4440
gttcgcgacg	ccccagaccg	atggatgacc	aacgcccgcg	tgaccactca	tcaaagcctg	4500
cttctcacag	agagggctac	gttcgctcca	ccagccgcgc	tcacacctgc	cactctctctg	4560
cttgaagaga	ctgatgaacc	agtgactcat	gattgccatc	aactattgat	tgaggagact	4620
ggggctccga	aggaccttac	agacataccg	ctgactggag	aagtgttaac	ctggttcaact	4680
40gacgggaagca	gctatgtggt	ggaaggtaag	aggatggctg	gggcggcggt	ggtggacggg	4740

acccgccagca tctgggccag cagcctgccg gaaggaactt cagcacaaaa ggctgagctc 4800
 atggccctca cgcaagcttt gcggctggcc gaagggaagt ccataaacat ttatacagac 4860
 agcaggtatg cctttgcgac tgcacacgta cacggggcca tctataagca aagggggttg 4920
 cttacctcag cagggaggga aataaagaac aaagaggaaa ttctaagcct attagaagcc 4980
 5ttacatttg caaaaaggct agctattata cactgtcctg gacatcagaa agccaaagat 5040
 cccatatcca gagggaaacca gatggctgac cgggttgcca agcaggcagc ccagggtggt 5100
 aaccttctgc ctatgataga aacacccaaa gccccagAAC cggagcaga gtacacccta 5160
 gaagactggc aagagataaa aaagatagac cagttctctg agactccgga agggacctgc 5220
 tatacctcag atgggaagga aatcctgccc cacaagaag ggtagaata tgtccaacag 5280
 10atacatcgtc taaccacct aggaactaaa cacctgcagc agttggtcag aacatctcct 5340
 tatcatgttc tgaggctacc aggagtggct gattcgggtg tcaaacactg tgtgccctgc 5400
 cagctgggtta atgctaattc ttccagaata cctccaggaa agagactaag gggaagccac 5460
 ccaggcgctc actgggaagt ggacttcact gaggtaaagc cggctaaata cggaacacaa 5520
 tatctattgg tttttgtaga caccctttca ggatgggtag aggcttatcc tactaagaaa 5580
 15gagactcaaa ccgtgggtggc taagaaaaaa ctggaggaaa tttttccaag atttgaata 5640
 cctaaggtaa tagggctcaga caatgggtcca gctttcgttg cccaggtaa gtcaggactg 5700
 gccaaagatat tgggattga ttgaaaactg cattgtgcat acagacccca aagctcagga 5760
 caggtagaga ggaatgaatg aaccattaaa gagaccctta ctaaattgac cgcggagact 5820
 ggcgttaaat attggaatg tctcctgccc tttgtgctt ttagggttag gaacaccctt 5880
 20ggacagtttg ggtcagcccc ctatgaata ctctacgggg gaccccccc attggtagaa 5940
 attgtctcog tacatagtgc tgacgtgctg ctttcccage ctttgttctc taggctcaag 6000
 gcacttgagt gggtagagca acgagcgtgg aggcaactcc gggaggccca ctcaggagga 6060
 ggagacttgc agatcc 6076

25

<210> 22

<211> 4918

<212> DNA

30<213> Porcine endogenous retrovirus

<400> 22

tggattttgc agactgtgga tcccgggggt tgcgacctta gcagccccac tctacccact 60
 aaccaaagaa aaaggggaat tctcctgggc tctctgagcag cagaaggcat ttgatgctat 120
 35caaaaaggcc ctgctgagcg cactgtctct ggcctctcct gacgtaacta aaccctttac 180
 cctttatgtg gatgagcgta agggagttag cggggaggtt ttaacccaaa ccttaggacc 240
 atggaggaga cctgtctcct accgtcctaa gaagctcgat cctgtagcca gtgggtggcc 300
 cgtatgcctg aaggctatcg cagctgtggc catactggtc aaggacgctg acaaatgac 360
 tttgggacag aatataactg taatagcccc ccattgcgtg gagaacatcg ttcggcagcc 420
 40cccagaccga tggatgacca acgcccgcgt gaccactat caaagctcgc tctcacaga 480

gagggtcacg	ttogetccac	cagccgctct	caacctctgc	actcttctgc	ctgaagagac	540
tgatgaacca	gtgactcatc	attgccatca	actattgatt	gaggagactg	gggtccgcaa	600
ggaccttaca	gacataccgc	tgactggaga	agtgttaacc	tggttcactg	acggaagcag	660
ctatgtgggt	gaaggtaa	ggatggctgg	ggcgccgtg	gtggacggga	ccgcacgat	720
5ctgggccagc	agcctgcggc	aaggaaactc	agcacaaaag	gctgagctca	tggccctcac	780
gcaagctttg	cggtggtccg	aagggaatac	cataaacatt	tatacagaca	gcaggatgac	840
ctttgcgact	gcacacgtac	acggggccat	ctataagcaa	aggggtgtgc	ttacctcagc	900
agggagggaa	ataaagaaca	aagaggaaat	tctaagccta	ttagaagcct	tacatttgcc	960
aaaaaggcta	gctattatca	actgtcctgg	acatcagaaa	gccaaagatc	ccatatccag	1020
10aggggaaccg	atggctgacc	gggttgccaa	gcaggcagcc	cagggtgtta	accttctgcc	1080
tatgatagaa	acacccaag	ccccagaacc	cggacgacag	tacacctgat	aagactggca	1140
agagataaaa	aagatagaac	agttctctga	gactccggaa	gggacctgct	atacctcaga	1200
tgggaaggaa	atcctgcccc	acaaagaagg	gttagaatat	gtccaaacaga	tacatcgtct	1260
aaccaccta	ggaactaaac	acctgcagca	gttggtcaga	acatctcctt	atcatgttct	1320
15agggctacca	ggagtggctg	attcggtggt	caaacactgt	gtgcctctgc	agctggttaa	1380
tgctaactct	tccagaatac	ctccaggaaa	gagactaagg	ggaagccacc	caggcgctca	1440
ctgggaagtg	gacttcactg	aggtaaagcc	ggctaaatac	ggaacaaaat	atctattggt	1500
ttttgtagac	accttttcag	gatgggtaga	ggcttatcct	actaagaaag	agacttcaac	1560
cgtggtggct	aagaaaatac	tggaggaaat	ttttccaaga	tttgaataac	ctaaggtaat	1620
20agggtcagac	aatggtccag	ctttcgctgc	ccaggtaagt	cagggactgg	ccaagatatt	1680
ggggattgat	tggaaactgc	attgtgcata	cagaccccaa	agctcaggac	aggttagagag	1740
gatgaataga	accattaaag	agacccttac	taaattgacc	gcggagactg	gcgttaatga	1800
ttggatagct	ctcctgcect	ttgtgctttt	tagggtagg	aacaccctgt	gacagtgttg	1860
gctgaccccc	tatgaattac	tctacggggg	acccccccca	ttggtagaaa	ttgcttccgt	1920
25acatagtgtc	gactgtgtgc	tttcccagcc	tttgttctct	aggctcaagg	cacttgagtgt	1980
ggtgagacaa	cgagcgtgga	ggcaactccg	ggaggcctac	tcaggaggag	gagacttgca	2040
gatcccacac	gctttccaat	tgggagattc	agctctacgt	agacgccacc	gtgcaggaaa	2100
cctcgagact	cggtggaagg	gccttatca	cgtacttttg	accacaccaa	cggtgtgtaa	2160
agtgaagga	atctccacct	ggatccatgc	atccacggtt	aagccggcgc	caacctccga	2220
30ttccgggtgtg	aaagccgaaa	agactgaaaa	tccccttaag	cttcgcctcc	atcgctgtgt	2280
tccttactct	gtcaataact	cctcaagtta	atggtaaacg	ccttggtggc	agcccgaact	2340
cccataaacc	cttatctctc	acctggttac	ttactgactc	cggtagaggt	attaattata	2400
acagcactca	aggggaggct	cccttgggga	cctggtggcc	tgaattatat	gtctgccttc	2460
gatcagtaat	ccctggtctc	aatgaccagg	ccacaccccc	cgatgtactc	cggtcttacg	2520
35ggttttacct	ttgcccagga	cccccaata	atgaagaata	ttgtggaat	cctcaggatt	2580
tccttttgca	gcaatggagc	tgcgtaactt	ctaattgatg	gaattggaaa	gtgccagctc	2640
ctcagcaaga	cagagtaagt	tactcttttg	ttacaatacc	taccagttat	aatcaattta	2700
attatggcca	tgggagatgg	aaagattggc	aacagcgggt	acaaaaagat	gtacgaaata	2760
agcaataaag	ctgtcattcg	ttagacctag	attacttaaa	aataagtttc	actgaaaaag	2820
40gaaaacaaga	aaatattcaa	aagtgggtaa	atggtatgtc	ttggggaata	gtgtactatg	2880

gagggctctgg gagaaagaaa ggatctgttc tgactattcg cctcagaata gaaactcaga 2940
 tggaaacctcc ggttgctata ggaccaata aggggtttggc cgaaccaagga cctccaatcc 3000
 aagaacagag gccatctcct aacctctctg attacaatac aacctctgga ctagtcccca 3060
 ctgagcctaa catcactatt aaaacagggg cgaacctttt taacctctac cagggagctt 3120
 5ttcaagctct taactccacg actccagagg ctacctcttc ttgttggtt tgcttagctt 3180
 cgggccacc ttactatgag ggaatggcta gaggagggaa attcaatgtg acaaaaggaac 3240
 atagagacca atgtacatgg ggatcccaaa ataagcttac ctttactgag gtttctggaa 3300
 aaggccactg cataggggat gttccccat cccaccaaca ctttgtaac cacactgaag 3360
 cctttaatcg aacctctgag agtcagtatc tggtagctgg ttatgacagg tggtagggcat 3420
 10gtaataactgg attaacccct tgtgtttcca ccttggtttt caaccaaact aaagactttt 3480
 gcgttatggt ccaaattgtc ccccggtgt actactatcc cgaaaaagca gtccttgatg 3540
 aatatgacta tagatataat cggccaaaaa gagagcccat atccctgaca ctagtgttaa 3600
 tgctcggatt gggagtgggt cgagcgctgg gaacagggaac ggctgcctc atcacaggac 3660
 cgcaacagct ggagaaagga cttagtaacc tacatcgaat tgtaacggaa gatctccaag 3720
 15ccctagaaaa atctgtcagt aacctggagg aatccctaac ctcttatct gaagtgggtc 3780
 tacagaacag aaggggggta gatctgttat ttctaaaaga aggaggatta tgtgtagcct 3840
 tgaaggagga atgctgtttt tatgtggatc attcaggggc catcagagac tccatgaaca 3900
 agcttagaga aaggttggag aagcgctgaa gggaaaagga aactactcaa ggggtggttg 3960
 agggatgggt caacagggtc ccttggttgg ctacctact ttctgcttta acaggaccct 4020
 20taatagtcct ctcctgttta ctacagttg ggcatgtat tattaacaag ttaattgcct 4080
 tcattagaga acgaataagt gcagtcaga tcatgggtact tagacaacag taccaaagcc 4140
 cgtctagcag agaagctggc cgctagctct accagttcta agattagaac tattaacaag 4200
 agaagaagtg gggatgaaa ggaatgaaaat gcaacctgac tctccagaa cccagggaag 4260
 taataagaag ctctaaatgc cctcgaatc cagacctgt tccctatagg taaaagatca 4320
 25tactttttgc tgttttaggg ctgtcttct gctctgtaca aaactttgtg gaaggggaaa 4380
 aacaggcccc tgagtattgt cctctatgct tgaaccttct tgaacctgct cctaactgct 4440
 tgttttgctt ctgtaaacct gcttgcatga gataaaaaa ggaagaagtc attgcctaac 4500
 ggaccctcct aagatcgggt gtaccacaaa atgttgaaac acatatctgt gtgacaacat 4560
 gtctcccca ccccgaaaca tgcgcaaatg tgtaactcta aaacaattta aattaattgg 4620
 30tccacgaagc gcgggctctc gaagttttaa attgactggt ttgtgatatt ttgaaatgat 4680
 tggtttggtaa agcgggggct ttgttgtgaa ccccataaaa gctgtcccga ctccacactc 4740
 ggggcgcgac tcctctaccc ctgctgggtg tagactgtgt ggcccagcg cgttggaat 4800
 aaaaatcctc ttgtgtttg catcaagacc gcttctctgt agtgattaag gggagtcgcc 4860
 ttttccgagc ctggagggtc tttttgctag tcttacagca cctttatttt ttccattt 4918

35

<210> 23

<211> 7873

<212> DNA

<213> Porcine endogenous retrovirus

40

<400> 23						
ggatctgttg	gtttctgttt	tgtgtgtctt	tgtcttgtgc	gtccttgtct	acagtttttaa	60
tatgggacag	acggtagcga	ccctcttagt	tttgactctc	gaccattgga	ctgaagttaa	120
atccagggct	cataaattgt	cagttcaggt	taagaaggga	ccttggcaga	ctttctgtgt	180
5ctctgaatgg	cgcacattcg	atgttggatg	gccatcagag	gggaccttta	attctgagat	240
tatectggct	gttaaagcaa	ttatttttca	gactggaccc	ggctctctac	ccaatcagga	300
gcctataatc	cttacgtggc	aagatttggc	agaggtacct	cgcctatggg	ttaaaccttg	360
ctggaataag	ccaagaaagc	caggtccccg	aattctggct	cttgagagaga	aaaacaaaca	420
ctcggtgcaa	aaagtcaagc	ctctctctca	tatetacccc	gagattgagg	agccgccggc	480
10ttgttcggaa	ccccaatctg	ttccccacc	cccttatctg	gcacagggtg	ctgcgagggg	540
acctctgcc	ctctctggag	ctccggcggt	ggagggaacct	gctgcaggga	ctcggagccg	600
gagggggcgc	accccggaag	ggacagacga	gatcgcgaca	ttaccgctgc	gcacgtacgg	660
ccctcccaca	ccgggggggc	aattgcagcc	cctccagtat	tggccctttt	cttctgcaga	720
tctctataat	tggaaaaacta	accatcccc	tttctcggag	gatccccaac	gcctcacggg	780
15gttggtggag	tccttatgt	tctctacca	gcctacttgg	gatgattgtc	aacagctgct	840
gcagacactc	ttcacaccg	aggagcgaga	gagaattctg	ttagaggcta	gaaaaaatgt	900
tctctggggc	gacggggcag	ccacgcagtt	gcaaaatgag	attgacatgg	gatttccctt	960
gactcgcccc	ggttgggact	acaacacggc	tgaaggtagg	gagagcttga	aaatctatcg	1020
ccaggctctg	gtggcggggc	tccggggcgc	ctcaagacgg	cccactaatt	tggctaaggt	1080
20aagagaagtg	atgcagggac	cgaatgaacc	ccctctgttt	ttccttgaga	ggctcttggg	1140
agccttcagg	cggtaacacc	cttttgatcc	cacctcagag	gccccaaaaa	cctcagtggc	1200
tttggccttt	ataggacagt	cagccttggg	tattagaaag	aagcttcaga	gactggaaag	1260
gttacaggag	gctgagttac	gtgatctagt	aaaggaggca	gagaaagtat	attacaaaag	1320
ggagacagaa	gaagaagggg	aacaaagaaa	agagagagaa	agagaggaaa	gggaggaaaag	1380
25acgtaataaa	cggcaagaga	agaatttgac	taagatcttg	gctgcagctg	ttgaagggaa	1440
aagcaataacg	gaagaagaga	gagattttag	gaaaatttag	tcaggcccta	gacagtcagg	1500
gaacctgggc	aataggaccc	cactcgacaa	ggaccaatgt	gcataattga	aagaaaaagg	1560
acactgggca	aggaactgcc	ccaagaaggg	aaacaaagga	ctgaaggctc	tagctctggg	1620
agaagataaa	gactagggaa	gacgggggtc	ggacccctct	cccagaccca	gggttaacttt	1680
30gaaggtggag	gggcaaccag	ttgagttcct	ggttgatacc	ggagcgaaac	attcagtgct	1740
actacagcca	ttaggaaaaa	taaaagataa	aaaatcctgg	gtgatgggtg	ccacaggggc	1800
acaaactgat	ccatggacta	ccgaagaac	agttgacttg	ggagtgggac	gggtaaccga	1860
ctcgtttctg	gtcatacctg	agtgccacg	acccctctta	ggtagagact	tattgaccaa	1920
gatgggggca	caaatctctt	ttgaacaagg	gaaacagaaa	gtgtctgcga	atacaaaacc	1980
35tatcactgtg	ttgacctctc	aattagatga	cgaatatcga	ctatactctc	ccctagtaaa	2040
gctgatcaaa	aataatacaat	tctggttggg	acagtttccc	caagcctggg	cagaaaccgc	2100
aggggatgggt	ttggcaaagc	aagttcccc	acaagttatt	caactgaagg	ccagtgccac	2160
accagtgta	gtcagacagt	accccttgag	taagaagact	caagaaggaa	ttcgcccgca	2220
tgtccaaaga	ttaatccaac	agggcatcct	agttcctgtc	caatctccct	ggaatactcc	2280
40cctgctaccg	gttagaaaac	ctggacttaa	tgactatcga	ccagtaacag	acttgagaga	2340

gggtcaataaa	cgggtgcagg	atatacacc	aacagtcctc	aacctttata	acctctgtg	2400
tgctctccca	ccccaacgga	gctgggtatac	agttatggac	ttaaaggatg	cctttttctg	2460
cctgagatta	cacccacta	gccaaacct	ttttgccttc	gaatggagag	atccaggtag	2520
gggaagaacc	gggcagctca	cctggaccgc	actgccccaa	gggttcaaga	actccccgac	2580
5catctttgac	gaagccctac	acagagacct	ggccaacttc	aggatccaac	acctcagagt	2640
gacctctctc	cagtacgtgg	atgacctgct	tctggcggga	gccaccaaac	aggactgctt	2700
agaaagcacg	aaggcactac	tgctggaatt	gtctgacct	ggctacagag	cctctgctaa	2760
gaaggccag	atgtgcagga	gagaggtaac	atacttggg	tacagtttgc	gggacgggca	2820
gcgatggctg	acggaggcac	ggaagaaaac	tgtagtcag	ataccggccc	caaccacagc	2880
10caaacagtg	agagagttt	tggggacagc	tggattttgc	agactgtgga	tccccggggt	2940
tgcgacctta	gcagccccac	tctaccact	aaccaaagaa	aaaggggaat	tctcctgggc	3000
tctctgcac	cagaaggcat	ttgatgctat	caaaaaggcc	ctgctgagcg	cacctgctct	3060
ggcctccct	gacgtacta	aacctttac	cctttatgtg	gatgagcgta	aggagtagtc	3120
ccggggagtt	ttaaacccaa	ctctaggacc	atggaggaga	cctgttgctt	acctgtcaaa	3180
15gaagctgat	cctgtagcca	gtggttggcc	cgtagcctg	aaggctatcg	cagctgtggc	3240
catactggtc	aaggacgctg	acaaattgac	ttgggacag	aataaactg	taatatgccc	3300
ccatgcgttg	gagaacatcg	ttcggcagcc	ccagaccga	tgtagacca	acgcccgcac	3360
gacctactat	caaagcctgc	ttctcacaga	gagggtcacg	ttcgtccac	cagccgctct	3420
caacctgtcc	actctctgc	ctgaagagac	tgatgaacca	gtgactcatg	attgccatca	3480
20actattgatt	gaggagactg	gggtccgcaa	ggaccttaca	gacataccgc	tgactggaga	3540
agtgttaacc	tggttcaactg	acggaagcag	ctatgtagt	gaaggtaaga	ggatggctgg	3600
ggcgcggtg	gtggacggga	ccgcacgat	ctgggcagc	agcctgcgcg	aaggaaacttc	3660
agcacaaaag	gctgagctca	tggccctcac	gcaagcttg	cggtggccg	aagggaaatc	3720
cataaacatt	tatacagaca	gcaggtatgc	ctttgcgact	gcacacgtac	acggggccat	3780
25ctataagcaa	aggggggtgc	ttacctcagc	agggaaggaa	ataaagaaca	aagaggaaat	3840
tctaagccta	ttagaagcct	tacatttggc	aaaaaggcta	gctattatac	actgtcctgg	3900
acatcacaaa	gccaaagatc	ccatatccag	agggaaaccg	atggctgacc	gggttgccaa	3960
gcaggcagcc	caggggtgta	acctctctgc	tatgatagaa	acacccaaag	ccccagaacc	4020
cggacgacag	tacaccctag	aagactggca	agagataaaa	aagatagacc	agttctctga	4080
30gactccggaa	gggacctgct	atacctcaga	tgggaaggaa	atcctgcccc	acaagaagg	4140
gttagaatat	gtccaaacga	tacatcgtct	aacctaccta	ggaactaaac	acctgcagca	4200
gttggtcaga	acatctcctt	atcatgttct	gaggctacca	ggagtggctg	atcggtgggt	4260
caaacactgt	gtgcctctgc	agctgggtaa	agccggctaa	atacggaaac	aaatatctat	4320
tggtttttgt	agacaccttt	tcaggatggg	tagaggctta	tctactcaag	aaagagactt	4380
35caaccgtggt	ggcttagaaa	atactggagg	gaaattttct	caagatttgg	aatacctaag	4440
gttaatgggt	cagacaatgg	tccagcttct	gttgccagag	taagtcaagg	actggccaag	4500
atattgggga	ttgattggaa	actgcattgt	gcatacagac	cccaaagctc	aggacagcta	4560
gagaggatga	atagaacctat	taaagagacc	cttactaaat	tgaccgcgga	gactggcggt	4620
aatgattgga	tagctctctc	gccctttgtg	ctttttaggg	ttaggaacac	ccctggacag	4680
40tttgggctga	ccccctataa	attactctac	gggggacccc	ccccattggt	agaaattgct	4740

tccgtacata	gtgctgacgt	gctgctttcc	cagcctttgt	tctctaggct	caaggcactt	4800
gagtggttga	gaccaacgagc	gtggaggcaa	ctccgggagg	cctactcagg	aggaggagac	4860
ttgcagatcc	cacatcgatt	ccaagtggga	gattcagctc	acgttagagc	ccaccgtgca	4920
ggaaacctcg	agactcgggt	gaagggccct	tatcacgtac	ttttgaccac	accaacggct	4980
5gtgaaagtcg	aaggaaatctc	cacctggatc	catgcatccc	acgttaagcc	ggcgccacct	5040
cccgattcgg	ggtggaaaagc	cgaaaagact	gaaaaatccc	ttaagcttcg	cctccatcgc	5100
gtgggtctct	actctgtcaa	taactcctca	agttaatggg	aaacgccttg	tggacagccc	5160
gaactcccat	aaacccctat	ctctcacttg	gttacttact	gactccggta	caggattata	5220
tattaacagc	actcaagggg	aggctccctt	ggggaccttg	tggcctgaat	tatatgtctg	5280
10ccttcgatca	gtaatccctg	gtctcaatga	ccaggccaca	ccccccgatg	tactccgtgc	5340
ttacgggttt	tacgtttgcc	caggaccccc	aaataatgaa	gaatatttgt	gaaatcctca	5400
ggatttcttt	tgcaagcaat	ggagctgcgt	aacttctaatt	gatgggaatt	ggaaaatggcc	5460
agtctctcag	caagacagag	taagttactc	ttttgttaac	aatcctacca	gttataatca	5520
atttaattat	ggccatggga	gatggaaga	ttggcaacag	cggttacaaa	aagatgtacg	5580
15aaataagcaa	ataagctgtc	attcgttaga	cctagattac	ttaaaaaata	gtttcactga	5640
aaaaggaaaa	caagaaaata	ttcaaaagtg	ggtaaatggg	atgtcttggg	gaatagtgtg	5700
ctatggaggc	tctgggagaa	agaaaaggatc	tgttctgact	attcgcctca	gaatagaaac	5760
tcagatggaa	cctcgtgttg	ctataggacc	aaataagggt	ttggccgaac	aaggacctcc	5820
aatccaagaa	cagaggccat	ctcctaacc	ctctgattac	aatacaacct	ctggatcagt	5880
20ccccactgag	cctaacaatca	ctattaaaa	aggggcaaaa	cttttaaac	tcattcaggg	5940
agcttttcaa	gctcttaact	ccacgactcc	agaggctacc	tcttcttgtt	ggctttgctt	6000
agcttcgggc	ccaccttact	atgagggaat	ggctagagga	gggaaattca	atgtgacaaa	6060
ggaacataga	gaccaatgta	catggggatc	ccaaaataag	cttaccctta	ctgaggtttc	6120
tggaaaaggc	acctgcatag	ggatggttcc	cccatcccac	caacaccttt	gtaaccacac	6180
25tgaagccttt	aatcgaacct	ctgagagtca	gtatctggta	cctggttatg	acaggtgggtg	6240
ggcatgtaat	actggattaa	ccccttgtgt	ttccaccttg	gttttcaacc	aaactaaga	6300
ctttgtgctt	atggtccaaa	ttgtccccgc	gggttactac	tatcccgaaa	aagcagctct	6360
tgatgaatat	gactatagat	ataatcgccc	aaaaagagag	cccataatcc	tgactatgac	6420
tgtaatgctc	ggatggggag	tggctgcagg	cgtgggaaca	ggaacggctg	ccctaatacac	6480
30aggaccgcaa	cagctggaga	aaggacttag	taacctacat	cgaattgtaa	cggaaagatct	6540
ccaagcccta	gaaaaatctg	tcagtaacct	ggaggaatcc	ctaacctcct	tatctgaagt	6600
ggttctcaag	aacagaaggg	ggttagatct	gttatttcta	aaagaaggag	gattatgtgt	6660
agccttgaag	gaggaatgct	gtttttatgt	ggatcattca	ggggccatca	gagactccat	6720
gaacaagcct	agagaaaagga	ctggagagcg	cccgcggttc	tcgaacaacc	cagacaggtt	6780
35gcttgtttca	attaagaagc	tgtcgaagta	accgctgagc	taaagccagc	ttagagaaag	6840
gttggagaag	cgtcgaaggg	aaaaggaaac	tactcaagag	tgggttgagg	gattggtcaa	6900
cagggtcctc	tgggtggcta	ccctactttc	tgctttaaca	gggaccttaa	tagtctcctc	6960
cctgttactc	acagttgggc	catgtattat	taacaagtta	attgccttca	ttagagaacg	7020
aatagtgca	gtccagatca	tggtacttag	acaacagtac	caaagcccg	ctagcagaga	7080
40agctggccgc	tagctctacc	agttcttaaga	ttagaactat	taacaagaga	agaagtgggg	7140

26

```

aatgaaagga tgaaaatgca acctgactct cccagaaccc aggaaggtaa taagaagctc 7200
taaatgcctt cgaattccag accctgttcc ctataggtaa aagatcatac tttttgctgt 7260
tttagggctt gctttctgct ctgtacaaaa ctttgtggaa ggggaaaaac agggccctga 7320
gtatgtgctt ctatgcttga aacttcttga aactgctcct aactgcttgt ttggccttctg 7380
5taaacctgct tgcataagat aaaaagagga gaagtcaatt gcctaacgga ccccgtaag 7440
atcgggtgta ccacaaaatg ttgaaacaca tatcttgggtg acaacatgtc tccccacccc 7500
cgaaacatgc gcaaatgtgt aactctaaaa caatttaaat taattgggtc acgaagcgcg 7560
ggctctcgaa gttttaaatt gactgggttg tgatatatttg aaatgattgg ttgttaaagc 7620
gcgggctttg ttgtaaaccc cataaaagct gtcccgaact cacactcggg gccgcagtcc 7680
10tctaccctg cgtggtgtac gactgtgggc cccagcgcgc ttggaataaa aatcctcttg 7740
ctgtttgcat caagaccgct tctcgtgagt gattaagggg agtcgccttt tccgagcctg 7800
gaggttcttt ttgctagtct tacaacagca cctcagtttt gttcctaaga agtctgcggc 7860
cctcaccag tca 7873

```

15

<210> 24

<211> 20

<212> DNA

20<213> Rous sarcoma virus

<400> 24

gggacgaggt tatgcgcgtg 20

25<210> 25

<211> 20

<212> DNA

<213> Rous sarcoma virus

30<400> 25

gggcgtgcgc gcattaccac 20

<210> 26

<211> 20

35<212> DNA

<213> Rous sarcoma virus

<400> 26

gaccgaccca gggaacaatc 20

40

27

<210> 27
<211> 20
<212> DNA
<213> Rous sarcoma virus
5
<400> 27
atgaggaaaa ttgcgggtgg 20

<210> 28
10<211> 20
<212> DNA
<213> Artificial Sequence

<220>
15<223> A primer

<400> 28
ggaatgtgac tggtaatgga 20

20<210> 29
<211> 20
<212> DNA
<213> Artificial Sequence

25<220>
<223> A primer

<400> 29
gccttagtga tggatggtg 20
30
<210> 30
<211> 21
<212> DNA
<213> Mus musculus
35
<400> 30
ccatccgtct tcatcttccc t 21

<210> 31
40<211> 21

<212> DNA

<213> Mus musculus

<400> 31

5tgggtcggtg tcctttagt t

21

<210> 32

<211> 6076

<212> DNA

10<213> Porcine endogenous retrovirus

<400> 32

ggagtttgag	ttttatcgaa	tttgaacag	tggtttacat	ggagattgta	gtgaaaggat	60
gaaaatgcaa	ctgactctc	ccagaacca	ggaagttaat	aagaagctct	aaatgccttc	120
15gaattccaga	ccctgttccc	tataggtaaa	agatcatact	ttttgctgtt	ttagggcttg	180
ctttctgctc	tgtacaaaac	tttgtggaag	gggaaaaaca	ggccctctgag	tatgtgcctc	240
tatgcttgaa	acttcttgaa	actgctccta	actgcttggt	tggtctctgt	aaacctgctt	300
gcataagata	aaaagaggag	aagtcaattg	cctaacggac	cccagtaaga	tcgggtgtac	360
cacaaaatgt	tgaaacacat	atcttgggtg	caacatgtct	ccccacccc	gaaacatgcg	420
20caaatgtgta	actctaaaac	aatttaaatt	aattgggtcca	cgaagcgcgg	gctctcgaag	480
ttttaaaattg	actggtttgt	gatattttga	aatgattggg	ttgtaaagcg	cgggctttgt	540
tgtaaacccc	ataaaaagctg	tcccgaactcc	acactcgggg	ccgcagtcct	ctacccctgc	600
gtggtgtacg	actgtgggccc	ccagcgcgct	tggaaataaaa	atcctctctgc	tgtttgcctc	660
aagacgcgct	ctcgtgagtg	attaagggga	gtcgcctttt	ccgagccttg	aggttctttt	720
25tgctagtctt	acatttgggg	gctcgtccgg	gatctgtcgc	ggccaccctc	aacacccgag	780
aaccgacttg	gaggtaaaaa	ggatcctctt	tttaactgtg	atgcatgtac	cgggccggctg	840
ctctgtcttg	agtgctctgt	ttcagtggtg	cgcgctttcg	gtttgcagct	gtcctctcag	900
accgtaagga	ctgggggact	gtgatcagca	gacgtgctag	gaggatcaca	gggtgccacc	960
ctgggggacg	ccccgggagg	tggggagagc	cagggaacgc	tggtggtctc	cttctgtcgg	1020
30tcagaggacc	gagttctgtt	gttgaagcga	aagcttcccc	ctccgcggcc	gtccgactct	1080
tttgctgctt	tgtggaagac	gcggacgggt	cgcggtgtgc	tggatctgtt	ggtttcttgt	1140
ttgtgtgtct	ttgtctctgt	cgctctgtgc	tacagtttta	atatgggaca	gacggtgacg	1200
acccctctta	gtttgactct	cgaccattgg	actgaagtta	aatccagggc	tcataatttg	1260
tcagttcagg	ttaagaaggg	accttggcag	actttctgtg	tctctgaatg	gccgacattc	1320
35gatgttgat	ggccatcaga	ggggaccttt	aattctgaga	ttatcctggc	tggtaaagca	1380
attatttttc	agactggacc	cggctctcat	cccaatcagg	agccctatat	ccttacgtgg	1440
caagatttgg	cagaggatcc	tccgccatgg	gttaaacctt	ggctgaataa	gccaaagaaag	1500
ccaggtcccc	gaattctggc	tcttgagag	aaaaacaac	actcggctga	aaaagtaag	1560
ccctctcttc	atatcatccc	cgagattgag	gagccgcgg	cttgcccgga	accccaatct	1620
40gttccccac	cccttatctt	ggcacagggt	gctgcgaggg	gacctctgc	ccctcttgga	1680

gctccggcgg	tggaggagacc	tgctgcaggg	actcggagacc	ggaggggcgcc	caccccgag	1740
gcgacagacg	agatgcgcgac	attaccgcgtg	cgacagtagc	gcctccccc	accggggggc	1800
caattgcagc	ccctccagta	ttggcccttt	tctctgcag	atctctataa	ttggaaaact	1860
aaccatcccc	ctttctcgga	ggatccccc	cgctccacgg	ggttgggtgga	gtcccttatg	1920
5ttctctcacc	agcctacttg	ggatgattgt	caacagctgc	tgacagacact	cttcacaacc	1980
gaggagcgag	agagaattct	gttagaggct	agaaaaaatg	ttcctggggc	cgacggggca	2040
cccacgcagt	tgcaaaatga	gattgacatg	ggatttccct	tgactcgccc	cgggtgggac	2100
tacaaacacg	ctgaaggtag	ggagagcttg	aaaatctatc	gccaggctct	gggtggcggt	2160
ctccggggcg	cctcaagacg	gccactaat	ttggctaagg	taagagaagt	gatgcaggga	2220
10ccgaatgaac	ccccctctgt	tttctctgag	aggtctctgg	aagccttcag	gcggtacacc	2280
ccttttgatc	ccacctcaga	ggcccaaaaa	gcctcagtg	ctttggccct	tataggacag	2340
tcagccttgg	atattagaaa	gaagcttcag	agactggaag	ggttacagga	ggctgagtta	2400
cgtgatctag	tgaaggaggc	agagaaaagta	tattacaaaa	gggagacaga	agaagaaaagg	2460
gaacaaagaa	aagagagaga	aagagaggaa	agggaggaaa	gacgtaataa	acggcaagag	2520
15aagaatttga	ctaagatctt	ggctgcagtg	gttgaaggga	aaagcaatac	ggaaagagag	2580
agagatttta	ggaaaaatag	gtcaggccct	agacagtcag	ggaacctggg	caataggacc	2640
ccactcgaca	aggaccaatg	tgcatattgt	aaagaaaaag	gacactgggc	aaggaaactgc	2700
cccaagaagg	gaacaaaagg	actgaaggct	ttagctctgg	aagaagataa	agactaggga	2760
agacgggggt	cggaacccct	ccccgagccc	agggtaactt	tgaagggtga	ggggcaacca	2820
20gttgagtccc	tggttgatcc	cggagcgaaa	cattcagtcg	tactacagcc	attaggaaaa	2880
ctaaaagata	aaaaatcctg	ggtgatgggt	gccacagggc	aaacaacagta	tccatggact	2940
accggaagaa	cagttgactt	gggagtggga	cgggtaaccc	actcgtttct	ggtcataact	3000
gagtgcccg	caccctctct	aggttagagac	ttattgacca	agatggggagc	acaaatttct	3060
tttgaacaag	ggaiaaccga	agtgctcgca	aataacaaac	ctatcactgt	gttgaccctc	3120
25caattagatg	acgaatatcg	actatactct	cccttagtaa	agcctgatca	aaatatacaa	3180
ttctgggttg	aacagtttcc	ccaagcctgg	gcagaaaacc	cagggatggg	tttggcagaag	3240
caagctcccc	caacagttct	tcaactgaag	gccagtgcca	caccagtgct	agtcagcagc	3300
taccctctga	gtaaaagaag	tcaagaagga	atcggccgc	atgtccaaa	attaatccaa	3360
cagggcatac	tagttctcgt	ccaatctccc	tggataactc	ccctgtctac	ggtagaaaag	3420
30cctgggacta	atgactatcg	accagtagac	gacttgagag	aggtcaataa	acgggtgcag	3480
gatacacacc	caacagtcct	gaacccttat	aacctctgtg	gtgctctccc	accccaacgg	3540
agctgggtata	cagttattgga	cttaaaagat	gcctttttct	gcctgagatt	acacccactg	3600
agccaaccac	tttttgccct	cgaatggaga	gatccaggta	cgggaagaac	cgggcagctc	3660
acctggagcc	gactgcccc	agggttcaag	aactccccga	ccatctttga	cgaagcccta	3720
35cacagagacc	tggecaactt	caggatccaa	caccctcagg	tgaccctctc	ccagtagctg	3780
gatgacctgc	tctctggcgg	agccacccaa	caggactgtc	tagaaggcac	gaaggcacta	3840
ctgtcggaat	tgctgcacct	aggctacaga	gcctctgcta	agaaggccca	gatttgacag	3900
agagaggtaa	catactgggg	gtacagtttg	cgggacgggc	agcgaaggct	gacggaggca	3960
cggaagaaaa	ctgtagtcca	gataccgggc	ccaaccacag	ccaacaagt	gagagaattt	4020
40ttggggacag	ctggattttg	cagactgtgg	atccccgggt	ttgcgacctt	agcagcccca	4080

ctctaccac	taaccaaga	aaaagggaa	ttctcctggg	ctcctgagca	ccagaaggca	4140
tttgatgcta	tcaaaaaggc	ctgctgagc	gcacctgctc	tggccctccc	tgacgtaact	4200
aaaccttta	ccctttatgt	ggatgagcgt	aaggagtag	ccggggaggt	tttaacccaa	4260
actctaggac	catggaggag	acctgttgcc	tacctgtcaa	agaagctcga	tcctgtagcc	4320
5agtggttggc	ccgtatgcct	gaaggctatc	gcagctgtgg	ccatactggt	caaggacgct	4380
gacaaattga	ctttgggaca	gaatataact	gtaatagccc	cccatgcgtt	ggagaacatc	4440
gttcggcaga	ccccagaccg	atggatgacc	aacgcccgcg	tgacccta	tcaagcctg	4500
ctctccacag	agagggtcac	gttcgtccca	ccagccgctc	tcaacctcgc	cactcttctg	4560
cctgaagaga	ctgatgaacc	agtactcat	gattgccatc	aactattgat	tgaggagact	4620
10ggggtccgca	aggaccttac	agacataccg	ctgactggag	aagtgttaac	ctggttcact	4680
gacggaagca	gctatgtggt	ggaaggtaa	aggatggctg	ggcgccggtg	ggtggacggg	4740
acccgcacga	tctgggcca	cagcctgcgc	gaaggaaact	cagcacaaa	ggctgagctc	4800
atggcccca	cgcaagcttt	gcggctggcc	gaagggaat	ccataaacat	ttatacagac	4860
agcaggatg	cccttgcgac	tgacacgta	cacggggcca	tctataagca	aagggggttg	4920
15cttacctcag	cagggaggga	aataaagaac	aaaggagaaa	ttctaagcct	attagaagcc	4980
ttacatttgc	caaaaaggct	agctattata	cactgtcctg	gacatcagaa	agccaaagat	5040
ccatatacca	gaggaacca	gatggctgac	cgggttgcca	agcaggcagc	ccagggtggt	5100
aaccttctgc	ctatgataga	aacacccaaa	gcccagaac	ccggacgaca	gtacacccta	5160
gaagactggc	aagagataaa	aaagatagac	cagttctctg	agactccgga	agggaacctgc	5220
20tatacctcag	atgggaagga	aatcctgccc	cacaaagaag	ggttagaata	tgtccaacag	5280
atacatcgtc	taaccacact	aggaactaaa	cacctgcagc	agttggtcag	aacatctcct	5340
tatcatgttc	tgaggctacc	aggagtggct	gattcgggtg	tcaaacactg	tgtgccctgc	5400
cagctggtta	atgctaatac	ttccagaata	cctccaggaa	agagactaag	gggaagccac	5460
ccaggcgtc	actgggaagt	ggacttcact	gaggtaaagc	cggctaaata	cggaaacaaa	5520
25tatctattgg	ttttttaga	caccttttca	ggatgggtag	aggcttatcc	tactaagaaa	5580
gagacttcaa	ccgtggtggc	taagaaaata	ctggaggaaa	tttttccaag	atttgggaata	5640
cctaagctaa	tagggtcaga	caatggtcca	gctttcgttg	cccaggtaa	tcagggactg	5700
gccaagatat	tggggattaa	ttggaaactg	cattgtgcat	acagacccca	aagctcagga	5760
caggtagaga	ggatgaatag	aaccattaaa	gagaccccta	ctaaattgac	cgcggagact	5820
30ggcgtaaatg	attgatatgc	tctcctgcc	tttgtgcttt	ttaggggtag	gaacacccct	5880
ggacagtttg	ggctgaoccc	ctatgaatta	ctctacgggg	gaccccccct	attggtagaa	5940
atgctctccg	tacatagtgc	tgacgtgctg	ctttccacgc	cttgtttctc	taggctcaag	6000
gcacttgagt	gggtgagaca	acgagcgtgg	aggcaactcc	gggaggccta	ctcaggagga	6060
ggagactcgc	agatcc					6076

United States Patent & Trademark Office
Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

☐ Page(s) _____ of _____ were not present
for scanning. (Document title)

Scanned copy is best available. Drawing some figures dark